

How to manage a peaceful world?

With luck, and chiefly because of Mr Mikhail Gorbachev's persistence, there is now a chance that peace will break out. Will the United States, the Soviet Union and their allies be able to manage the transition?

WYOMING, a remote but attractive state, has been in its time one of the most substantial bases for US ballistic missiles, but last weekend's meeting there between the US Secretary of State, Mr James Baker, and his opposite number from the Soviet Union, Mr Eduard Shevardnadze, could well hasten the end of the state's strategic importance. The crucial development is the Soviet Union's now-declared willingness not to make the abandonment of the Strategic Defense Initiative (SDI) a condition of a treaty to limit the deployment of strategic weapons, while insisting that the United States must agree to the strict interpretation of the Anti-Ballistic Missile (ABM) Treaty by which the two superpowers undertook not to "develop, test or deploy" ABM systems based at sea or in space. On the face of things, the US administration is prepared to accept this limitation; Baker would have said so were it not. So there is a substantial prospect of an agreement at some stage next year that Soviet and US forces of strategic weapons will be reduced by half. By any tests, that will be well worth waiting for.

But that seems not to be the end. President George Bush's plan to announce this week a new development towards a treaty to ban the manufacture of chemical weapons has been well flagged, and could imply more good news ahead (see page 271). And the British government, among others, has been making cheerful noises about the likelihood of reasonably quick progress in the negotiation at Vienna of a treaty on conventional weapons in Central Europe. The only obviously missing ingredient is a test-ban treaty. Only a little reflection will conclude that there has not so far this century been a time when there was such a substantial promise that arms-control agreements would not be ends in themselves but means towards the more enduring end of reducing, perhaps even eliminating, the risk of another world war. Even the rash of treaties agreed between 1968 and 1972 could not be said to have accomplished that.

Most of the credit for these developments must go to Mr Mikhail Gorbachev, who has often bemused his counterparts in the West with proposals for arms-control agreements at once daring and challenging. It is easy to forget that it is only a few years since Gorbachev at Helsinki almost persuaded his US counterpart, President Ronald Reagan, that the time had come to get rid of strategic weapons altogether. But, recognizing that it takes two (at least) to reach an agreement, Gorbachev

might have talked until he was blue in the face. What has made the difference, this time, is *glasnost*, conceivably conceived of as an instrument of social equity (free speech) or, more deviously, as a means by which local and regional party chiefs within the Soviet Union might be confronted with their constituents' complaints, which has turned out to be a way of signalling that the superpower Soviet Union has paid a bankrupt's price to remain a superpower: there are missiles in the bunkers, but there is no butter in the shops.

Soviet citizens (in whichever Soviet republic they live) should acknowledge how temperate has been the response. At no stage in the four months just past, made particularly informative by the first session of the new Supreme Soviet (otherwise the Congress of People's Deputies), has there been a Western voice of substance demanding armed incursion (or even subversion) of the East; the flimsy doctrine that it is safest to attack an adversary when he says he is flat on his back appears to have been forgotten. There are two explanations, of which the full bunkers are the most immediate. The other is that the West is genuinely at a loss to know how to accommodate the certain prospect of fluidity in the East. Of course, it is no surprise that even Western sophisticates have lost the habit of living without national chauvinism.

Euphoria, the known enemy of realism, must also be contained. What this weekend's meeting in Wyoming confirmed is simply that the US administration is prepared to let SDI wither on the vine; the research will continue, but under other line items of the US defence budget. It will probably be the same in the Soviet Union, empty supermarket shelves notwithstanding. But both superpowers will be helped if the cost of military procurement can be reduced. That is Gorbachev's declared intention, but Bush should have similar objectives. Both should also acknowledge that their agreement, by proxy, in Wyoming does not legislate for other governments, those of South-east Asia, the Indian subcontinent and the Middle East (not to mention China and even Latin America). Who will be allowed to inspect those missile sites and weapons production plants?

There is also a more subtle social difficulty to be resolved: people in the West may welcome, for example, Moldavian linguistic nationalism as a sign that the empire in the East is in the process of collapse, just as people in the Soviet Union may applaud the Irish Republic Army



(which killed eleven British Marine bandsmen last week) as a bunch of freedom fighters. But, in reality, neither side would welcome the reality of those movements in the world that the arms-control agreements now in prospect are likely to create. The trick they must next pull off is to share the other's view of what constitutes good government. Necessarily, that will be more difficult than mere arms control. □

Defensive business

The failure of a British company's investment in a US business should be followed by more general reform.

SINCE the beginning of the year, the British Ministry of Defence has been brooding on the question of whether the General Electric Company (no relation of GE) and Siemens of West Germany should be allowed to buy the electronics, telecommunications and defence company called Plessey. Throughout, the ministry has been comforted by knowing that even if two of its chief electronics contractors merged, competition would remain because of the continued independence of Racal and Ferranti, the second of which, despite its reputation for daring, has a solid reputation in avionics. But no longer. Hardly had Plessey been taken over than Ferranti was in trouble.

The circumstances are bizarre, but also raise important questions about the role of modern contractors. Ferranti's troubles stem from its purchase more than a year ago (for £360 million) of the US defence contractor International Signal and Control (ISC). Ferranti's objective was to buy its way into a more substantial (and presumably more profitable) defence business than the British government offered. Eighteen months went by before it emerged that ISC does not, as it had claimed, enjoy the benefits of contracts worth between £150 million and £200 million with governments outside the United States. Ferranti's bankers have bridged the gap, but only temporarily; eventually, it will probably be sold.

The important public issue is that ISC appears not to have been one company, but two. Like all other public companies, it had a publicly acknowledged board of directors. But because much of its work was technically secret, there was a parallel 'proxy' board, one of whose members was (and still is) the much-respected ex-admiral "Bobby" Inman. The idea seems to have been that the latter, with the benefit of the appropriate security clearances, would talk technicalities to the Pentagon and other customers, presumably directing technical operations as well, while the public board would worry about matters that more usually concern directors — cash flow, the share price, corporate and public relations. Nobody (not even Ferranti) seems to have appreciated that such a division of responsibility, itself a violation of the principles of a joint-stock company, is also a recipe for not being able to tell who is responsible when things go wrong. It is a pity that Ferranti should have had to pay such a heavy price for demonstrating such a simple truth. □

We're all Greens now

If British Greens in conference last week are anything to go by, the Green movement has a problem of consistency.

LARGELY because of the continuing schism between the two British centre parties, the British Green party did better than anybody (itself included) expected at the British elections to the European Parliament in the summer. It is, of course, a familiar *canard* to say that Greens do not know what they want: the truth is that their wants are clearly articulated. But the British Green party's conference last week at Wolverhampton (a distant north-western suburb of Birmingham) seems merely to have confirmed that Greens' wants are a self-contradictory collection of yearnings for a different world that cannot constitute a political programme, but which may yet win votes.

Last week's conference reached largely predictable decisions. Nuclear power stations, for example, were condemned. So, too, was nitrate in drinking water, as were pesticides anywhere. Congestion on the roads could be made to disappear by levying sufficiently heavy charges on the vehicles using them. And so on. As the week went by, it became plain that it is unfair to call the British Green party a single-issue party; rather, it is a multi-issue party, an umbrella beneath which the discontents of all individual members can be accommodated. It is a party of the malcontent. Comparisons with the defunct French Poujadistes (who wanted not to pay taxes) are natural, and not misplaced.

It will be a great misfortune if the new 1990s' wave of environmentalists is too much enmeshed in doings such as these. In an environment more beneficent (at least for people) than ever before, there remain a few important environmental problems (such as AIDS and the possibility that the greenhouse effect will take effect) and a multitude of environmental threats less damaging to people (but not necessarily less threatening to other species), most of them consequences of human activities made possible by increased prosperity. Last week's Greens seem not to have bothered with the distinction.

In reality, all electable political parties have to square a tricky circle: they have to persuade those who vote for them that certain goals (AIDS and the greenhouse effect, say) are paramount and that others are matters on which people must be prepared to compromise. It is, for example, entirely possible that future governments in Europe and North America will find themselves having to relax drinking water standards so as to pay for capital investments needed to combat the greenhouse effect. And how much will Greens be prepared to pay to buy out last week's declaration by four southern African governments that they will continue to allow the trade in elephant ivory? Faced with such dilemmas, on last week's showing, the British Green party would promptly fall apart. □

Row over controversial new AIDS drug

- Doctors enraged by *ad hoc* trial
- Benefits claimed by patients

San Francisco

A HIGHLY controversial 'clandestine' study indicates that the promising AIDS drug Compound Q is not the cure many had hoped but, rather, one potentially useful — but extremely toxic — weapon in the growing arsenal being brought to bear against the disease.

The San Francisco advocacy group Project Inform established the trial last May after *in vitro* studies showed that Compound Q selectively killed cells infected with HIV, the virus causing AIDS. Organizers argued that patients had already begun to taking the drug on their own initiative and that federal safety and efficacy trials were proceeding too slowly. The *ad hoc* trial outraged many doctors and researchers, who felt that it undermined traditional science. And last week's announcement of the first results further emphasized the split over the issue.

Project Inform co-director Martin Delaney received a standing ovation when he proclaimed at a public meeting: "We have reported faster over this protocol than any research in the history of the epidemic". But Professor Jerome Groopman of Harvard Medical School condemned the effort. "This is an important and a very bad precedent", he said. "This is not the way we are going to identify effective treatments for AIDS." The trial's most conclusive data concerned the toxicity of the drug, which is derived from a cucumber-like plant and which has been used in China to treat cancer and induce abortions. Almost all the 51 patients in San Francisco, New York and Florida developed side-effects, ranging from fever and rash to violent dreams and coma. Three patients died after taking Compound Q, but researchers said all were in advanced stages of the disease and that no direct relationship could be established between their deaths and the drug.

On the positive side, many patients showed signs of benefiting from Compound Q. The Florida group was dropped from all except the toxicity data because doctors there used a wide range of dosages and administration methods that made comparisons impractical. But the 34 patients in San Francisco and New York generally showed an increase in the count of CD4 cells, which are depleted by HIV infection. Researchers were encouraged by several other findings. One involved P24 antigens, high levels of which are typically associated with an advanced stage of AIDS. Results of this test were announced

only for the 19 San Francisco patients: 15 showed a decline and nine showed a sustained drop averaging 50 per cent. In addition, 12 of the San Francisco patients reported feeling more energetic than they had in months.

Nine gained weight and seven said they were mentally sharper. While trial organizers stressed that Compound Q is not a cure for AIDS, they insisted that it shows promise — especially if used with other drugs, such as AZT, which interfere with HIV's ability to infect new cells. Dr Alan Levin, who helped to organize the trial, said, "I believe that in combination with other drugs it has the potential for cure". Soon after the trial became public, the US Food and Drug Administration (FDA) launched an investigation, which is still continuing. Delaney claims that the trial is legal because it was established as a "treatment protocol" between doctors and existing patients, rather than a clinical study. Nevertheless, Project Inform took elaborate precautions to warn patients of the risks involved, both to assuage FDA fears and to avoid lawsuits.

A lawyer and a patient advocate were on hand as each subject signed the informed consent agreement — and the entire process was videotaped.

But opponents were unconvinced. "Martin Delaney and Al Levin are not competent to do experimental drug trials", said Groopman, who said he supports community-based research but not when it involves a highly toxic drug used for the first time in humans against a particular disease. He added: "It's quite likely patients unnecessarily suffered". Groopman attacked the trial on other grounds, challenging the protocol and criticizing the absence of a neutral third-party review. Delaney said trial results will be independently evaluated. But, in general, project organizers took a hard line with critics. Levin noted that patients often experience severe side-effects during FDA-approved trials. The difference, he said, is that the Compound Q results were quickly made public.

Besides, community physicians know best what is good for their patients, "Academic physicians are not qualified to care for the chronically ill", he added.

While the debate is yet to be resolved, more traditional work continues. An FDA-approved Phase I trial of Compound Q began in May at San Francisco General Hospital. And the National Institute of Allergy and Infectious Disease (NIAID)

RADIOCARBON LABORATORIES

Blind dating exposes UK weakness

London

BRITAIN'S radiocarbon-dating laboratories are to launch an international project to try to overcome a lack of consistency in their results. A workshop held at the National Engineering Laboratory (NEL) in Kilbride, Scotland, heard last week that a comparison of 40 radiocarbon-dating laboratories in Britain found a disturbing pattern of errors.

Three Scottish laboratories, the Statistics Department of the University of Glasgow, the Natural Environment Research Council (NERC) Radiocarbon Laboratory in East Kilbride and the Scottish Universities Research and Reactor Centre, had collaborated to design, execute and interpret a "blind intercomparison exercise" supported by the Science and Engineering Research Council (SERC).

In the trials, 38 laboratories were asked to date samples of wood, peat and carbonate. Professor Murdoch Baxter of NEL said the results were "gloomy". He said that only seven out of 38 laboratories had met the three desired performance criteria — negligible bias, high internal precision and negligible external variation. Radiocarbon dates were "two to three times less accurate than implied by their error terms". He said most laboratories produced differing dates when they analysed similar objects of the same age.

Baxter said that although delegates were "disappointed" by the results, there was a "positive determination to work together and improve the situation" and the carbon-dating community "would adopt quality assurance principles and practice". Each laboratory will improve monitoring and build up a "record of regular essays of in-house and externally supplied reference standard materials". The International Atomic Energy Authority will supply samples of wood, sediment and carbonate on request from 1990 with given dates to provide reference materials. "There are a lot of good laboratories and a lot of poor ones", Baxter said. "It is important there is more cooperation to allow the good laboratories to help the others." To monitor improvements, a second blind check will be carried out in two years with the help of the NERC.

Ben Webb

will soon announce funding awards under its new Community Program for Clinical Research in AIDS, designed to bolster community involvement in research. Said Daniel Hoth, director of NIAID's Division of AIDS, "Our view is that there's enormous energy and enthusiasm among community physicians to do clinical trials, and we want to help them channel that to productive research that can help find out which drugs work and which don't".

Robert Buderl

False accusations at London hospital

London

FALSE accusations of fraud have been levelled at a British medical scientist at a London teaching hospital, apparently in an attempt to damage his reputation. The person accused (whose name is being deliberately withheld) holds appointments as a lecturer at the Royal Postgraduate Medical School (RPMS) and as a registrar at the Hammersmith Hospital, London, where the school is based.

One bizarre feature of the case is that letters to the school and to the Wellcome Trust, from which the scientist is seeking a scholarship, said that *Nature* had been conducting an investigation of several improprieties, including the forging of references in an application for registration to the General Medical Council and plagiarism of the work of other researchers in scientific papers submitted for publication. The letters are signed "John Dickson, for the Vanguard Detective Agency", and are constructed so as to suggest that the detective agency is employed in the investigation.

But in a letter to *Nature*, "John Dickson, PhD" merely suggests that an investigation should be carried out on the basis of the same and further allegations, including collusion with a travel agent to convert overseas travel funds into cash. There is no record of Vanguard Detective Agency, but the address in suburban west London from which the letters were sent is adjacent to an engineering workshop labelled "Vanguard Engineering Company".

Further allegations include the assertion that the accused scientist may have "fraudulently obtained his medical degree, that he was dismissed from a PhD course at Brunel University and that 'the testimony of his fellow-workers' reveals that his conduct as a scientist is unethical. But the records office at Brunel University says there is no record of the accused scientist ever having been a student there.

Officials of the RPMS say they have the highest regard for the person accused, believing that he is at the start of a distinguished career. A senior member of the staff there says he has known the person's family for 30 years, and that the letters concerned must be a cruel hoax.

In the event, the author of the letter appears to be somebody who also holds an appointment at the Hammersmith Hospital. Earlier this week, he was under pressure to resign. An official of the Wellcome Trust said that the accusing letter might have been taken seriously, "and we might have found ourselves writing an unfortunate letter", had it not been for the "fishy" coincidence that the address given for the Vanguard Detective Agency is in the street in which the accused scientist lives.

Ben Webb

Battle over value of words

Washington

THE law courts of France, West Germany, Switzerland and the United States will provide the unlikely setting next year for a battle between two US publishers over the value for money offered by scholarly journals in physics. The battle began with a survey of the cost-effectiveness of physics journals published in the *Bulletin of the American Physical Society* and, in shortened form, in *Physics Today*. The commercial publisher Gordon and Breach, which emerged as the least cost-effective of those surveyed, claims that Henry Barschall, the author of the survey, was biased and the methodology was flawed. It is taking legal action against the American Physical Society (APS) and the American Institute of Physics (AIP) — the parent organizations of the two journals which published the survey — and against Barschall, in European countries where relevant laws are strict.

Barschall, a retired professor of physics at the University of Wisconsin in Madison, was prompted to examine the value for money of physics journals because their rising costs were forcing the university physics library to cancel subscriptions (see this issue page 349). He surveyed more than 200 physics journals, rating them according to cost and impact. Journals of both the APS and the AIP emerge among the most cost-effective.

Barschall measured cost-effectiveness as the ratio of the cost per printed character of a journal to the frequency with which it is cited, and found dramatic variations in the results. In the field of condensed-matter physics, for example, he reports that the cost-effectiveness varies by a factor of 200. "All the publishers whose journals have low average costs per character or low ratios of cost to impact are scientific societies or associations, while the publishers whose journals have high costs per character or high ratios of cost to impact are commercial firms." Barschall concludes that a solution to the present financial "crisis" in libraries caused by increasing costs of journals might be for authors to publish their papers in journals with low cost per character. In the survey, Gordon and Breach had the highest average ratio of cost to impact: 29.5 compared to 0.28 for the APS and 1.1 for the AIP, one of its member societies.

The lawyer representing Gordon and Breach in Switzerland, Alfred Gilgan, says this gives the impression that Gordon and Breach is a company of "crooks who are extorting their subscribers". The company has filed suits against APS, AIP and Barschall, seeking retraction, damages and an injunction preventing further articles comparing the physics journals of both publishers until the

dispute is resolved. AIP offered to settle the dispute by publishing a response from Gordon and Breach in *Physics Today*, but this was rejected because the response was to be published along with a letter from Barschall.

Gilgan maintains that the article in *Physics Today* is a "disguised" advertisement for AIP and APS. "Barschall is not an independent scientist who conducted a survey in the interest of the science community", he says, claiming that Barschall is biased because of his links with AIP. For two years before he wrote the article, he was on the governing board of AIP. When he wrote the article, Barschall was still a member of the publishing policy committee of AIP, as was declared in a footnote to the article. Barschall denies the accusations of bias, saying that APS and AIP "had nothing to do with" the survey, and that he had no financial links with AIP or APS.

Gilgan also accuses Barschall of making "outrageous errors" in his calculations and using "misleading" methodology. The cost-per-character measure is too simplistic and "unjustly prejudices journals with a high content of formulae, graphs and charts", he says. Richard Meserve, one of the lawyers for AIP in the United States, disputes the claims, but admits that any method "is necessarily going to be imperfect". But Gilgan also argues that the costs calculated by Barschall for the Gordon and Breach journals were between 10 and 50 per cent too high, and accuses Barschall of choosing to survey the most expensive journals published by Gordon and Breach.

The measurement of 'impact' by a citations score is also criticized by Gilgan as a measure only of the circulation figure of a journal, not of its value to a subscriber. Highly specialized journals, such as those published by Gordon and Breach, have smaller circulation figures and thus higher production costs and subscription prices, he says, than general physics journals such as those of AIP. But "the characters that are actually useful to the subscriber are cheaper at Gordon and Breach than at AIP", he claims.

Barschall says that he used "standard" methodology and that he did not claim it was "the sole determinant as to what is a useful journal"; he wrote the article "from the point of view of a librarian" and "librarians need to know how much they have to pay to get a page of a journal". He also disputes that the journals of Gordon and Breach are more specialized than those of AIP and APS and that he selected those which are the most expensive. He surveyed, he says, all the physics journals to which he had "easy access" in the library.

Christine McGourty

SOVIET ARMED FORCES

Military hit by brain drain

London

THE Soviet armed forces are suffering from a temporary but acute brain-drain, Defence Minister General Dmitrii Yazov told an *Izvestiya* interviewer last week. The shortage, he said, is due to the early demobilization of students who had been called up for military service. Since for the most part student-draftees were assigned to jobs requiring a high level of technical expertise, their premature departure leaves serious gaps. More than 700 tanks and 900 infantry combat vehicles will be left without crews, and the rocket forces will have to go on to a watch-and-watch system instead of the present watch in three. It will take at least a year for the army and two years for the navy to make up the deficit.

The decision to release the students ahead of time came after several years' campaigning from rectors and faculty members of universities and higher education institutes who pointed out that national service interrupted a young man's career just when he was at the peak of intellectual creativity. Army service, they maintained, spoiled him for a scientific career, without for the most part making a good soldier out of him. The early demobilization of the students was widely hailed as the first step in the plan to reduce the Soviet armed forces by 500,000 men. Yazov now says, however, this is not so. The cuts, he explained, are being made by reducing the annual size of the draft

HIPPARCOS

Rescue nearly over

Munich

PRELIMINARY results obtained last week demonstrated that the European Space Agency (ESA) astrometry satellite Hipparcos will yield some data, if not as much as originally planned. The perigee of Hipparcos's still very elliptical orbit (see *Nature* 341, 3; 1989) has been increased to 526 km above the Earth's surface, and its rotation has been stopped. ESA was intending to remove the protective baffles from the telescope aperture on 26 September.

On-board tests have indicated that the telescope and its instrumentation appear to be working. Except during the satellite's passage through the Van Allen radiation belts, 5,000 km above the Earth, the telescope's response was as expected, suggesting that Hipparcos will be able to return useful data 90 per cent of the time. Once the baffles are removed, it will be four weeks before ESA can gauge accurately the length of the mission. The current best estimate is that Hipparcos will survive six months, compared with the planned duration of two years.

Steven Dickman

intake. During the past six months, since the announcement of the cuts, the armed forces have been reduced by 148,900 men.

This figure does not include the 175,700 students, of whom some 168,000 have already returned to their studies. Yazov did not specify the criteria for reducing the intake. Possibly it is being done by raising health requirements. During the past few years, there have been frequent

PRIZES

USA, Japan and UK share research award



Washington

Four scientists share this year's Albert Lasker Basic Medical Research award, which recognizes their four individual discoveries of intracellular signalling pathways. Edwin G. Krebs, of the University of Washington, discovered protein phosphorylation, a fundamental biochemical reaction responsible for the progress of many enzyme reactions; Michael J. Berridge, of the University of Cambridge, was responsible for understanding the importance of calcium in cell growth, and its regulation by inositol triphosphate; Yasutomi Nishizuki, of Kobe University, elucidated the role of the enzyme protein kinase c, important in the regulation of both normal and abnormal cell growth; and Alfred G. Gilman, of the University of Texas, discovered G-proteins, a class of proteins that convey information from cell-surface receptors to internal cell processes.

The 1989 Clinical Research Award goes to Etienne-Emile Baulieu of the University of Paris, for his lifelong studies of the biological synthesis and functions of steroid hormones. Baulieu is publicly known for his discovery of the abortifacient drug RU486, which works by blocking the receptors for progesterone.

Lewis Thomas, of Cornell University, adds the 1989 Lasker Public Service Award to a number of book awards, for *The Lives of a Cell* and *The Medusa and the Snail*, and numerous honorary degrees. The Lasker citation recognizes Thomas for his promotion of biomedicine and public health not only through his popular writings but also by his activities as an administrator and teacher. Above, from left to right: (top) Edwin Krebs; Michael Berridge; Yasutomi Nishizuki; (bottom) Alfred Gilman; Etienne-Emile Baulieu and Lewis Thomas.

David Lindley

complaints about the physique of recruits, of whom almost one in ten fails the medical examination.

The gaps left by the students must once again raise the question of whether it would be more efficient to replace compulsory military service by a smaller, highly trained, professional army.

In the meantime, while the politicians and high command argue costs, much of the Red Army and Navy's most sophisticated equipment is apparently lying idle.

Vera Rich

Avoiding imports in Japan

Tokyo

PLANS are afoot to drastically re-structure the blood-product industry in Japan to reduce dependence on imported blood products which were the main source of AIDS infection. But the re-structuring could result in increased prices for blood coagulants for haemophiliacs, the main victims of AIDS in Japan.

A report released this month by an advisory body to the Ministry of Health and Welfare, the New Blood Business Promotion Study Council, recommends that all production of blood coagulants should be transferred from the private sector to the Japan Red Cross by 1991 and that coagulants should be made only from domestic blood. Until now, all coagulants have been made by private companies

IVORY IMPORTS

Japanese importers slide under the wire

Tokyo

It seems to be impossible to stop Japan's ivory importers. In June, the Ministry of International Trade and Industry (MITI) announced a partial ban on ivory imports in the face of international criticism and concern about dwindling populations of elephants in Africa (*Nature* 340, 85; 1989). But no sooner was the partial ban announced than Japanese importers began stockpiling imported ivory on a large scale.

The stockpiling came to light after MITI announced on 13 September a "temporary" total ban on imports as part of a new import quota system intended to prevent exactly what has happened.

The earlier ban, affecting all imports of worked ivory, also banned indirect imports of raw ivory from suppliers in places such as Hong Kong and Singapore. Announced by MITI on 16 June, it took effect only three days later, but in the interval Japanese importers had placed orders in Hong Kong for about 20 tonnes of raw ivory, or about 20 per cent of Japan's annual use in recent years, according to Toru Takimoto of MITI's International Trade Administration Bureau.

Even after the partial ban took effect, direct imports from Africa continued, reaching by this month about 100 tonnes, or about as much as for the whole of last year. This stockpiling seems not to have been affected by the undertaking on 15 June to show "self-restraint by the several Japanese organizations involved in the import of ivory" (Japan General Merchandise Importers' Association, Japan Ivory Fine Arts and Crafts Cooperation Federation, Tokyo Ivory Fine Arts and Crafts Association and Osaka Ivory Fine Arts and Crafts Association).

The stockpiling has not yet shown up

using mostly cheap US blood plasma. The report also recommends that more domestic blood should be used in the production of other blood products, such as albumin and globulin, and a new public organization should oversee the blood business in Japan.

Japan has come under international criticism for its excessive imports of blood products. In 1975, the World Health Organization (WHO) recommended that blood products should be made from domestic blood supplies. But by the mid-1980s, Japan's imports of blood plasma had grown to occupy about a third of the world market, and domestic blood supplied only a few per cent of demand. Coincident with this surge in the use of imported blood, AIDS was introduced to

in import statistics because the ivory has been deliberately held in bonded warehouses so as not to appear in customs-cleared trade statistics. But international embarrassment will be unavoidable when the trade figures for August appear any day now, according to a well-informed source.

Japan imports almost half of the world's ivory. The industry is worth about ¥20,000 million (\$140 million) a year and supports about 30,000 employees. Over half of the imports go into the manufacture of pencil-sized personal seals that can cost as much as ¥80,000 (\$550) for a set of three. When the various ivory trade organizations pledged to show "self-restraint" in June, they also expressed their hope to continue their business no matter how "frugal" it might be.

The United States, European nations and several other countries have imposed total bans on ivory imports. And there are strong international pressures to impose a total import ban under the Convention on International Trade in Endangered Species (CITES) to which Japan is a party. A CITES meeting to vote on the issue will be held next month in Switzerland.

MITI said in July that while, "in principle", Japan favours a total ban, there are some countries (notably South Africa, Zimbabwe and Botswana) that wish to continue trade on a sustainable basis and "it is not proper to disregard their intention and deprive them of economic resources unilaterally". Takimoto says MITI will decide on the length of its present temporary total import ban after next month's CITES meeting. But critics say the temporary ban is just a "cosmetic" measure for the meeting in Switzerland.

David Swinbanks

Japan in non-heat-treated blood coagulants made from US plasma and more than 90 per cent of AIDS carriers in Japan are haemophiliacs who were treated with such products.

The Ministry of Health and Welfare has tried to reduce consumption of blood products by discouraging unnecessary prescription by doctors. Consumption of blood plasma dropped from a peak of 3.8 million litres in 1985 to 2.7 million litres in 1987 but has since risen to 2.8 million litres in 1988. On a *per capita* basis, this is still more than twice the consumption of the United Kingdom and France, for example (see *Nature* 322, 193; 1988).

The driving force behind blood consumption in Japan is huge discounting by pharmaceutical companies. The health and welfare ministry sets 'official' prices for drugs in April each year. But by using cheap imported blood plasma and blood products, companies have been selling blood products at far below the official price. Hospitals, which charge patients and the national health insurance system the official price, have been able to reap enormous profits by prescribing more and more blood products. The council report calls for production of cheaper domestic products to compete with imports. But experts express scepticism about the ability of the Red Cross to take over the blood coagulant business and produce cheap products.

A recent report in the Japanese newsletter *Nikkei Biotechnology* points out that the cost of blood plasma produced by the Japanese Red Cross (¥45 or out \$0.3 per millilitre) is five times that of imported plasma and about seven times the price of plasma in the United States. Part of the reason for the high cost is that about 80 per cent of the blood is collected by mobile blood donation vans which are not cost effective. Unless the efficiency of blood collection is improved, the cost of domestically produced blood products is unlikely to fall.

The *Nikkei Biotechnology* report also doubts whether the Red Cross will be able to produce all blood coagulants by 1991. Although Japan has one of the highest blood donation rates in the world, most of the blood is used for transfusions, leaving little for production of blood-plasma products. The council report calls for blood donations to be increased from about 1.9 million litres in 1988 to 2.3 million litres in 1991 to supply the blood-plasma necessary for domestic blood-coagulant production.

But this target is insufficient if conventional methods of blood-coagulant production are used. A new monoclonal-antibody technique developed by Baxter pharmaceutical company in the United States gives a higher yield of blood coagulant and could perhaps meet Japan's domestic needs. The council report calls

CHEMICAL WEAPONS

International forum backs ban

Canberra

A PROPOSED international ban on the production and stockpiling of chemical weapons could push up the worldwide price of chemicals by one-quarter per cent. According to Tom Reynolds, head of Australia's industry delegation to the international Government-Industry Against Chemical Weapons Conference held in Canberra last week, consumers would have to bear the \$1,000 million cost to industry of the new treaty.

The ban received the unanimous support of representatives of 95 per cent of the world's chemical industry, including those from Iraq and Iran, which were accused of using chemical weapons during the Gulf War. The aim of the conference was to bring government and industry together to discuss the practicalities of an international ban on chemical weapons.

Much of the proliferation in chemical weapons seen in the past decade has been linked to the production of chemicals by private industry, without governmental control. The chemicals that provide precursors to those used in weapons have a wide range of civilian applications.

Chemical weapons have been on the

for import of the latest foreign technology and, according to *Nikkei Biotechnology*, the Red Cross has already approached Baxter.

But in the United States, blood coagulants produced by the monoclonal-antibody technique are much more expensive than conventional coagulants and are beyond the reach of many haemophiliacs. Given the high price of blood plasma in Japan, it seems certain that the price of domestically produced coagulants will be high. Seizo Murakami, chairman of the blood business promotion council, says the council has "no idea" what the price of Red Cross coagulants will be.

Blood-product manufacturers refuse to comment on the report. But a representative of the Chemo-Sero-Therapeutic Research Institute (Kaketsuken), a manufacturer in Kyushu, at a press conference last year, complained bitterly that the Red Cross had made requests for transfer of its blood-product manufacturing technology. And, according to a reliable source, Kaketsuken is "furious" about the council's recommendations.

Mitsuru Miyata, editor of *Nikkei Biotechnology*, says that Japan's four blood-product manufacturers (Kaketsuken, Midori Juji, Fuji Rebio and Nihon Seiyaku) realize that blood products are unlikely to be profitable and are diversifying into new areas. But it seems unlikely that the Red Cross will be able to replace their expertise in research and development.

David Swinbanks

agenda in the Geneva arms-control negotiations since 1968. In Paris earlier this year, 149 nations met for a conference on the prohibition of chemical weapon use.

Four hundred delegates from 60 countries, excluding Libya and Syria which chose not to attend, were present in Canberra. The Soviet Union and the United States are the only two countries to admit to having chemical weapons, although another 20 countries are believed to have the capability to produce them. The United States is the only country that admits to producing chemical weapons.

The conference agreed to the formation of an industry forum to lobby in Geneva for an early signing of a treaty. Signing is unlikely to take place before 1992, but there was general agreement that political and technical issues could be worked out by the end of 1990.

Important elements of the proposed treaty will be a combination of regular and impromptu inspections of each participating nation's chemical plants. Concern over commercial confidentiality was partially allayed by a call for amendments to the treaty protecting the confidential transfer of data between industry and the national governments of the inspectors. "This will give industry some confidence that their trade secrets won't suddenly come out in the public domain", said Reynolds.

Challenge inspections would be conducted by an agency similar to the International Atomic Energy Agency, a multilateral organization that inspects nuclear power plants worldwide. Such a secretariat would cost \$100 million a year.

There was little agreement, however, on how to deal with countries that break the treaty. "In the end there is no international police force; if a country is determined to break its obligations, it can. If a signatory does not comply with the verification procedures outlined in the treaty, then it is no longer a party to it, and industry would suffer if it dealt with that country," said Michael Costello, delegation leader for Australia.

Both the Soviets and the Americans were eager to be the first to announce initiatives. The Soviets claimed to be the first to agree to the challenge inspections "anytime, anywhere". The US delegates countered with then-Vice President George Bush's 1984 speech urging impromptu inspections.

Nikita Smidovich, head of the Soviet arms control and disarmament section called the Americans "double-faced" because of their continued production of chemical weapons while advocating non-proliferation and an eventual ban. The United States ceased production of chemical weapons in 1969 and resumed in

1988, because its stockpile was no longer considered a credible deterrent to the Soviet Union, which did not cease production until 1987.

With the world's chemical industries opposing all "diversion of industry's products for the manufacture of chemical weapons", the US government may have to make its own chemicals to avoid forcing its industry to break the agreement. According to Kyle Olson, one of the US industry representatives, the Americans are getting at least some of their chemical-weapon products on the open market. The US ambassador to the Geneva weapons talks, Max Friedersdorf, said that as chemical weapons were being produced with congressional approval, the use of commercial products in chemical weapons did not constitute a "diversion of industry products."

Tania Ewing

... and so does Bush

Washington

IN his first speech before the United Nations General Assembly last Monday, US President George Bush proposed steps towards a worldwide ban on the production and stockpiling of all chemical weapons. The proposal followed a successful weekend meeting in Jackson Hole, Wyoming, between Secretary of State James Baker and Soviet Foreign Minister Eduard Shevardnadze, at which the United States and the Soviet Union agreed to provide information on their chemical-weapons arsenals and allow mutual inspection of storage sites.

Bush said that the United States is ready to destroy "98 per cent" of its chemical weapons stockpile in the first eight years of a new treaty, provided the Soviet Union joins the ban, and to destroy "every one" of its chemical weapons within ten years once all nations capable of producing chemical weapons sign the treaty.

A major new element of the proposal is the willingness of the United States to begin chemical weapons cuts before an international treaty is concluded. "The United States is ready to begin now", said Bush, and is prepared to eliminate "more than 80 per cent" of its chemical weapons before a treaty is signed, provided only that the Soviet Union reduces its stockpile to an equal level and there is agreement on the conditions, including inspection, under which stockpiles are to be destroyed.

The Bush proposal is partly seen as an attempt by the United States to regain the momentum lost to the Soviet Union in global arms-control negotiations. But the willingness of the United States to begin making cuts immediately is clearly intended to have a wider effect, pushing forward the 40-nation negotiations at Geneva on a chemical-weapons ban.

Alun Anderson

Candidates' science lessons

São Paulo

THE support of science and technology has not been a conspicuous topic in speeches by candidates in the Brazilian presidential election, due on 15 November. But several national institutions have submitted to the candidates a 45-point document reminding them of the importance of science to Brazil's development, and making suggestions for the future.

Although it is meant to be optimistic, the tone of the document gives a measure of the despair felt by the scientific community. It tells the candidates, for example, which government bodies deal with research funding, as if George Bush or Michael Dukakis had had to be told in last year's US election campaign what the National Science Foundation is and what it does.

The report comes from the federal government's Special Secretariat for Science and Technology, with the support of the Brazilian Academy of Science, the Council of Rectors of Brazilian Universities and the influential Federation of Industries of the State of São Paulo, the country's most industrialized state.

Notably absent from the signatories was the Brazilian Society for the Progress of Science (SBPC), the biggest association of scientists; its president, Ennio Candotti, said that even though he agrees with most of what the report says, it was not "politically convenient" to sign it, because of its governmental origins.

SBPC is unhappy with the present government's treatment of science. At the beginning of President José Sarney's term, a new Ministry for Science and Technology was created, and there was a promise to increase research and development spending to 2 per cent of gross national product (GNP). But with Sarney's term due to expire in March 1990, spending on research and development stands at only 0.7 per cent of GNP, and the Science and Technology Ministry has been replaced by the Special Secretariat for Science and Technology, a body with cabinet rank, but without the status of a ministry. But SBPC's criticisms are partially echoed in the document, which is why Candotti has not condemned it.

Thus the report admits some problems that scientists have been complaining of for years. Bureaucratic complexity in foreign currency exchange, along with laws restricting the import of items for

which a Brazilian equivalent exists, have made it almost impossible to make use of foreign funds for research. Brazil is now negotiating the second phase of a World Bank loan that would provide \$330 million for research, with an equal sum to be provided by the federal government, over the next five years. This would be an enormous boost for university research, but only if the money actually reaches scientists.

The suggestions in the document include new tax incentives for private companies investing in research and development, and better integration of university research with industry. This is a theme particularly dear to the secretary of science and technology, Décio Leal de Zagottis, former director of the Escola Politécnica, São Paulo's engineering school.

The initiative now rests with the presidential candidates. According to press reports, the leading candidate, Fernando Collor de Mello, plans to reduce the number of ministries from 23 to 10, but has apparently decided to retain the Special Secretariat for Science and Technology, rather than merging it with a bigger ministry. But Brazilian scientists will not lose sleep over the fate of a government department: what they fear most is Brazilian politicians' habit of dismantling the work of their predecessors, making empty any pretence of a long-term policy for research.

Ricardo Bonalume Neto

INDIA

Restriction venture

New Delhi

INDIA has launched its first venture capital company to make restriction enzymes. Bangalore Genei Private Ltd has been set up by Dr P. S. Babu, a theoretical physicist-turned-molecular biologist, and Dr K. Prasad, an immunologist, both of whom have been associated with the Indian Institute of Science, Bangalore. A large part of the funding for the company comes from the Technology Development and Information Company of India.

The new company will obtain the know-how for the manufacture of the enzymes from Astra Research Centre, Bangalore, which carries out research in medical biotechnology and is developing diagnostic kits for a variety of tropical diseases. The Centre for Genetic Engineering will also assist the new company.

Restriction enzymes are currently being imported by individual laboratories and in bulk quantities by the CSIR Centre for Biochemicals, New Delhi. The new company will initially make the more important and commonly used restriction enzymes.

K. S. Jayaraman

Observation satellite flies first hurdle

Paris

DESPITE financial worries within the member states of the European Space Agency (ESA), the next-generation European remote-sensing satellite (ERS-2) last week survived an early round of discussion of its fate. There was concern within ESA that the satellite would have to be cancelled or postponed, as its major backers — Britain, France and West Germany — were rumoured to be unenthusiastic. The three nations are expected to provide about 65 per cent of the costs. But at the Earth Observation programme board meeting last week, member states supported a recommendation that the satellite programme should begin.

In the corridors of ESA, reactions to the news are restrained, as the go-ahead for ERS-2 (or EEMS — European environment monitoring satellite as it is known within ESA) now depends on the decision of the ESA council at its October or December meeting. And it is only then, if a decision to proceed is agreed, that member states will be asked to commit themselves to a budget.

The uncertainty over ERS-2 has nevertheless emphasized budget difficulties within ESA. Having committed themselves in November 1987 to the costly Columbus programme, the Hermes space shuttle and Ariane-5 launcher, member states have little money left for the optional 'user programmes', such as Earth observation, and microgravity research. France, having agreed to finance its own Spot remote-sensing programme to the end of the century (see *Nature* 340, 583; 1989), was expected to give ERS-2 only a lukewarm reception. Similarly, West Germany agreed only a third of the increase requested in next year's space budget (see *Nature* 340, 179; 1989), putting both ERS-2 and the data-relay satellite, DRS, under threat.

The US Landsat and French Spot remote-sensing satellites do not have the same goals or capabilities as ERS, whose synthetic aperture radar allows images to be collected at night and through cloud cover, while a radar altimeter measures peaks and troughs in the ocean surface to within a few centimetres. ERS-2 should cost "about 51 per cent" of the total costs of ERS-1, according to a discussion document, and is needed to provide data continuity between ERS-1, due for launch in October next year and the polar platform scheduled for 1997. If ERS-2 does not go ahead immediately, say sources within ESA, not only will this continuity be lost, but the satellite will become more difficult and expensive to build if existing teams disband. A novel global-ozone measuring experiment it is to carry will also be threatened.

Peter Coles

Correction: German telescopes

There have been no funds set aside for the building of a large new telescope in West Germany, contrary to the first paragraph of an article in *Nature* last week (*Nature* 341, 177, 1989). One of the telescope designers mentioned is Gerhard Schnur of the University of Bochum. □

ANTARCTIC SCIENCE

Women accept polar challenge

Munich

IGNORING the example set by the US Antarctic research station and others, West Germany has always refused to send women to spend the winter at its own Antarctic station. Nine all-male teams in a row have overwintered at the station.

This autumn, that will change, when nine women will make the long trip from Bremerhaven to the Georg-von-Neumayer-Station on the Ekström shelf ice. The women, trained in survival skills, have important scientific tasks to perform during their 320-day period of isolation from February to December 1990.

Frailty was not the problem for German women researchers eager for duty in the Antarctic. Gotthilf Hempel, director of the West German polar research institute in Bremerhaven, never wanted to send a mixed team because of the risk of pregnancy.

For years, the leader of the current expedition, Monika Puskeppeleit, was frustrated by this rule. Ever since finishing her medical degree at Heidelberg in 1984, she had been prodding Hempel to let her join the West German team. In 1987, when she was about to give up and ask the Australians if she could join their team, Hempel agreed to consider another solution — sending a women-only team. "I am a biologist and I believe in fecundity", explained Hempel. "A pregnancy in the isolation of the Antarctic can be fatal for both mother and child."

The Neumayer Station is equipped with a makeshift operating room for emergency procedures such as appendectomies. But help from the outside would be impossible during most of the winter, when temperatures reach -46°C . Would Hempel not trust the researchers to avoid pregnancy in the Antarctic? "No", he said, "I do not trust [birth-control] procedures completely." Puskeppeleit and two geophysicists found two like-minded meteorologists and four technicians to complete the team. The team will measure seismic disturbances, as well as observing the Earth's magnetic field as part of the international project Intermagnet and collect data on the weather for global climate studies.

Puskeppeleit will gather data for a joint project with the German Aerospace Research Establishment (DLR). She will see if the bacterium *bacillus subtilis* is suitable as a "living sensor system" for measuring the effects of the seasonal stratospheric ozone deficiency in the Antarctic. Similar experiments with the bacterium have been carried out in space.

Unlike the larger US base at McMurdo, the German station is a temporary structure, consisting primarily of two large steel pipes. West Germany plans to invest in a permanent station to be built by the

early 1990s. The German station is located at $70^{\circ}37'$ south latitude, $8^{\circ}22'$ west longitude off the Weddell Sea. The nearest other station belongs to South Africa and is 200 km away.

Hempel said that if he had reservations about sending the all-female team, "I would not have let them go". They are not going purely for the adventure of it or just to show off, he said. Instead, "they take it as a natural right" that women should have an equal opportunity.

Steven Dickman



The nine West German women due to leave for a 14-month stay in the Antarctic this autumn.

France asks for advice

Paris

FRANCE, which is to host a meeting of Antarctic Treaty signatories on 19 October, is looking at alternatives to ratification of the Wellington Convention on the Regulation of Antarctic Mineral Resource Activities (CRAMRA). With only a few weeks left before signatories to the treaty have to make up their minds, the Parliamentary Office of Evaluation of Technological and Scientific Choices was asked to give its advice and organized a 4-day series of hearings open to the press.

The parliamentary office was set up in July 1983 and consists of eight senators and eight deputies whose task is to advise parliament on the consequences of scientific and technological policies. Their decision to invite a string of experts — from marine biologist, Commander Jacques Cousteau, and the ecology group Greenpeace to senior civil servants and the head of the national petrochemical institute — to state their points of view in front of the press had no precedent.

CRAMRA aims to close a legal loophole in the existing treaty by limiting mineral exploration on the continent (see *Nature* 341, 93; 1989). Until the convention comes into force, all 16 signatories are bound by a moratorium on mineral exploration, which expires in November. So far, France and Australia have refused to ratify CRAMRA, saying that it will encourage, rather than simply limit,

commercial exploration. Instead, they suggest that the Antarctic continent should be designated a world nature reserve, with only research activities authorized.

This position was last week endorsed by Remy Parmentier, of Greenpeace, and Cousteau, even though a ban on mineral exploration is unlikely to receive a unanimous vote by treaty signatories. The argument that the ban would be difficult to enforce, they say, also applies to the current moratorium. Lucien Montadert, director of the Institut Français du Pétrole, told the office that a search for oil in the Antarctic would not be technically feasible for 30 years, but added that, "if the possibility exists to look for oil, some day someone will look for it". Even if, for safety reasons, scientific geological surveys are carried out precisely where there is little likelihood of finding gas or oil, said Admiral Corbier, administrator of the French Antarctic Territories, the boundary between research and prospecting is often ill-defined.

Last Thursday, at the end of the session of expert testimony, it seemed likely that the office would recommend that France adopt a compromise position. Instead of ratifying CRAMRA, France will probably call for the existing moratorium to be extended until a competent authority is found to administer the Antarctic as a world nature reserve.

Peter Coles

Time for another change

Washington

US and world agriculture is ready for its third major change this century according to a report released by the US National Research Council, the research arm of the National Academy of Sciences. After the mechanization of agriculture, which began early in the century, and the introduction of chemical fertilizers, pesticides and high-yielding crop varieties which produced the 'green revolution' of the 1960s, will come 'alternative' farming that uses skilful management to take advantage of the biological relationships that occur naturally on the farm.

Alternative Agriculture is a 450-page report produced by a committee of 17 researchers from 11 US universities and is based on detailed studies of 14 US farms where alternative farming practices were being tried out. Although the title of the report suggests an association with the 'alternative society' of the 1960s, the report makes plain that "the hallmark of an alternative farming approach is not the conventional practices it rejects but the innovative practices it includes".

The overall objective is said to be to enhance and sustain useful biological relations rather than to reduce and simplify them through conventional monocultures and blanket application of pesticides and herbicides. As examples of the alternative approach the report notes:

- Genetic improvement of crops to resist pests diseases and drought, and to use nutrients more effectively.
- The planting of a diversity of crops rather than a single one. Crop rotation can reduce problems from weeds, disease and pests and cut fertilizer use and soil erosion.
- Reliance on the prevention of disease in

animals rather than frequent use of antibiotics.

■ Integrated pest control methods.

Integrated pest control uses ecological principles to capitalize on natural pest mortality, predator-prey relations, genetic resistance and the effect of the precise



timing of tilling, cropping, pruning and pesticide application. Typically, integrated pest management requires fields to be 'scouted' to determine pest and disease levels before deciding what form of minimal intervention is best. The overall effect can be to reduce pesticide use and to make the farm more productive.

John Pesek, chairman of the NRC committee and head of the department of agronomy at Iowa State University, argues that alternative agriculture is economically successful chiefly because it reduces the cost of "off-farm inputs" such as fertilizers and pesticides, while maintaining productivity.

"Successful alternative farmers do what all good managers do. They apply management skills and information to reduce costs and improve efficiency while maintaining yield levels", he says.

The report's good news is that alterna-

tive methods work and through reduced pesticide use and better soil management, could help to ameliorate conventional agriculture's problems — 3,000 million tons of topsoil erode from US farms each year, and pesticides and fertilizers in agricultural run-off, when added to the silt, are major causes of water pollution. But the bad news is that federal farming programmes make it very difficult to deviate from conventional farming practices. Seventy per cent of US cropland is covered by commodity programmes designed to shield farmers from fluctuating prices. One effect is that any practice that reduces acreage planted to a programme crop reduces the acreage eligible for subsidies for the next five years. Thus, if a farmer switches from corn to alfalfa for one year to increase nitrogen in the soil, he will incur an extended loss of subsidies.

Commodity programmes also base future payments on past production. While this system has helped to push up US agricultural production, it has also encouraged farmers to cultivate marginally productive land and to use levels of fertilizer and pesticide that would make little sense without incentives. The result, the report says, is that many farmers simply aim to "maximize present and future program benefits".

Other programmes encourage massive use of pesticides by setting cosmetic standards for fruit that have little bearing on nutritional quality. Tolerance of minor surface blemishes would permit major reductions in pesticide use.

The committee recommends that federal commodity programmes, which cost \$14,000 million a year, be restructured "to help farmers realize the full benefits of the productivity gains possible through many alternative practices". At a minimum, the programmes should be changed to encourage crop rotation.

The US Department of Agriculture (USDA) has generally welcomed the report and accepted that change is needed. But the challenge will be to provide farmers with the much higher level of training and information they need to adopt new practices. That help is not available from traditional sources such as USDA, extension agents or agricultural supply dealers. The report calls for a new \$40 million competitive grants programme for alternative agriculture research, along with increased funds for demonstration and implementation of the research results.

Pesek says he cannot predict the precise economic effects of the widespread use of alternative agriculture. But he says the committee is convinced the methods work, and that they would produce an ample food supply while reducing environmental problems. The potential benefits, he says, are "too attractive to continue to lie fallow".

Alun Anderson

BIOTECHNOLOGY LICENSING

EC announces bovine hormone moratorium

Munich

IN the middle of this month, European Agriculture Commissioner Ray McSharry made the long-expected announcement of a 15-month moratorium on the use of the genetically engineered hormone bovine somatotropin (BST). The moratorium is to allow for further study of the product. McSharry had been expected to propose a ban in August, but the Commission of the European Communities (EC) shied away in the face of pressure from the United States (see *Nature* 340, 415; 1989).

The moratorium (which the EC prefers to call a "period of evaluation") must still be approved by the Council of Ministers, which will meet soon to discuss the matter. The European Parliament has also been asked to comment. The commission will issue a report based on the studies by

October, 1990. A decision on licensing is expected to follow.

Clayton Yuetter, the US Secretary of Agriculture, has backed away from the threatening position he took in July when the moratorium was first discussed. He said he accepted the EC proposal as long as it was linked to scientific studies. But "a delay of 20 years" would not meet that criterion, he said.

US and EC officials are struggling to avoid a trade war over BST, which is expected to set a precedent for the licensing of other biotechnology products in Europe. Yuetter said that if the EC wants to reject BST on grounds other than efficacy, safety and quality — for example on economic grounds — then it should say so in the GATT international trade negotiations.

Steven Dickman

PhDs and the ESRC

SIR—Good graduate students publish — and the earlier they do so the better for science and themselves. Yet one public body in Britain, the Economic and Social Research Council (ESRC) has a policy which in effect prevents this.

The ESRC will not fund postgraduate studentships for academic departments where 60 per cent of PhDs are not completed within three years. (A year's grace is allowed for examiners to read the thesis and to arrange to meet.) The threat of blacklisting puts departments under strong pressure to discourage graduate students from publishing lest this delay them in finishing their theses.

In its evaluation, ESRC take account of all PhDs done in a department, so that its policy affects students who are self-financed or supported by other research councils. The policy is justified on grounds of increasing "productivity" of postgraduate education, but there is no evidence that its policy will increase the production of better scientists. Indeed, in its search for an administratively recognizable measure of the efficient use of resources, ESRC's policy may have the opposite effect. Creative scientists will be penalized in two ways. First, activities unrelated to PhD work, however important to later work, will tend now to be seen negatively.

Second, individuals showing traits of individuality and nonconformity at their interviews may be judged unlikely to complete in three years and so be denied admission.

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Peer review

SIR—Heartened by the recent comments on the role of journals in science (*Nature* 339, 657; 1989) and on anonymous peer review (*Nature* 340, 424; 1989) I would like to add the following points. They are not intended to be generalizations without exception; rather their validity depends on individual manuscripts.

The editor should check that the comments of the reviewers are based on a thorough examination of the manuscript. This is reasonable because the decision to publish rests solely with the editor and presumably is based largely on the reviewers' comments. Furthermore, the authors of the submitted manuscript should be informed of the reasoning behind the editor's decision, including which of the reviewers' comments were agreed or disagreed with and the relative importance given to these assessments. This will enable the authors of the manuscript to judge whether the editor's decision to

accept or reject has been based on an acceptable level of knowledge of the results and understanding of the interpretations.

Should there be any statements in a manuscript supported by 'data not shown'? Would it not be better for the excess data to be placed in an appendix which is subject to review and, after publication, available on request?

Although reviewers have a selfless, charitable and onerous task, they receive in return knowledge of results, techniques and concepts well in advance of others. To a reviewer who is a competitor of the manuscript's authors, this can be a very big advantage over the other, less privileged, competitors. This is particularly true for reviewers who are in charge or part of large groups.

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SIR—One step towards a solution of the problem of peer reviews is for referees to volunteer their identities to authors. Surely any referees who feel they are being fair-minded and objective have nothing to fear from such a disclosure?

Another step might be for manuscripts to be sent to a first referee for comment, then for the first referee's remarks as well as the manuscript to be sent to a second referee. In this way, the second referee would make a judgement not only of the manuscript but also of the first referee's comments.

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AIDS in Bulgaria

SIR—A recent article in *Nature* discussed the necessity of Bulgaria's mandatory programme for identifying people seropositive for the human immunodeficiency virus (HIV)¹. So far, more than two million people have been tested in Bulgaria, including so-called 'high-risk' groups (homosexuals, prostitutes, citizens who have spent time abroad and foreigners, including students who are long-term residents), as well as members of the general population aged 16–65 years.

Letters submitted for Correspondence should be typed, double-spaced, on one side of the paper only.

To determine previous exposure to HIV, individuals are screened with an enzyme immunoassay (EIA), and reactive samples are confirmed by the Western blot (immunoblot) procedure. Confirmed positive results usually indicate the presence of virus in the body. On the other hand, negative results by EIA do not necessarily indicate the absence of HIV-1 infection, as virus can be isolated and proviral sequences detected in infected individuals who remain seronegative for prolonged periods^{2,3}. By 7 July 1989, 77 Bulgarian citizens and 72 foreigners, 59 of whom were students, chiefly from Africa, were reported to be seropositive for anti-HIV by EIA (data supplied by Ministry of Health, Sofia). The seropositive students were sent home. Of the 77 EIA-positive samples from Bulgarian nationals, 53 were classified as indeterminate by Western blot⁴. Current experience indicates that virus will not be recovered from these people. So far in Bulgaria, only three people have been reported to the World Health Organization as definite AIDS cases⁵.

Mandatory serological testing is not unique to Bulgaria. For example, in Illinois, where mandatory premarital testing of 70,846 applicants for marriage licences was enforced, eight seropositives were obtained at a cost of \$312,000 per identified individual⁶. The screening data from Bulgaria, revealing 1.2 confirmed seropositives per 100,000 Bulgarian nationals, indicate that HIV is confined chiefly to certain high-risk groups. It is noteworthy that the three specimens positive for virus were from members of such groups. The overall results are most encouraging from a public health point of view and suggest that there should be a reevaluation of the mandatory programme. In arriving at a decision, financial considerations must be weighed against possible benefits.

It would be desirable, particularly for countries such as Bulgaria where the HIV epidemic is at a very early stage, to have international reference laboratories to turn to in order to verify the presence of HIV-1 by isolation or by determination of proviral sequences.

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Sunset for dangerous chemicals

SIR—Work on existing chemicals that are dangerous to man and/or the environment tends to focus on one issue at a time, at present chlorofluorocarbons (CFCs). For many years, polychlorinated biphenyls have been given the same intense attention. Plans to phase out these chemicals are under way. But such plans take a long time to develop, are very resource-demanding and may lead to development of alternatives that pose other threats.

In my view, however, the number of chemicals that need to be phased out is too large to allow time to proceed case by case. My suggestion is that we try to establish a process, nationally or, preferably within some international organization, such as the Organisation for Economic Development (OECD), whereby chemicals that need to be phased out (sunset chemicals), are identified according to generally accepted criteria.

The procedure might be as follows.

(1) Identify, according to set criteria, a number of chemicals (say 50 or 100) that should be phased out.

As a basis, lists of 'priority' chemicals, or of chemicals with specific hazardous properties — carcinogenic, mutagenic, teratogenic or hazardous to the environment — could be used. Criteria should be established whereby 'multiproblem' chemicals are distinguished. Step 1 would involve setting-up expert committees to develop criteria and the chemicals to be selected.

(2) Advertise the list, giving a proposed date for the phasing out of each chemical. At this stage, industry must be allowed to argue why a certain chemical should not be phased out. In Sweden this means that industry has to prove beyond reasonable doubt that the chemical in question is not a candidate for a sunset list.

(3) Once the list has been agreed to, nationally and/or internationally, companies could be obliged to report annually on the development of the phase-out. There should be a rule that no company must report a figure similar to or higher than that of the year before.

It is important to stress the quantitative aspect of the process because these are chemicals we want to get rid of. Companies may of course also report on protective measures, but this must not confuse the goal. The Swedish experience, for example with the Halving of Pesticides Programme, shows that such quantitative goals promote incentive and ingenuity.

(4) Results should be published regularly. On a national level, companies could compete, for example, for the title 'Company of the year'.

(5) The process should end with a ban, the sunset. Such a ban should be inherent in the process from the start. Responsible

companies will have developed alternatives or closed processes, while less responsible companies will be punished for not having done so. Discussions and agreements between governments and industry will be a normal part of the process.

Such a process may be started at a national level and implemented by national legislation and regulation. As most chemicals move extensively in international trade and thus appear in, for example, many OECD member countries, the process needs to be internationalized. And a common understanding of the process within the international community would be an important step towards greater co-operation and harmonization between countries. The OECD could set an example to the rest of the world and take a strong lead on the road to a sustainable future.

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Academic boycotts

SIR—Distinguishing academic from sports boycotts of South Africa ("Academics, cricket and apartheid" *Nature* 340, 413; 1989) does seem appropriate in the light of the vital need for Great Britain and other nations steadfastly to support the academic institutions in South Africa that will be so essential in buttressing a post-apartheid society. In modern history, universities have generally outlasted the institutions of government, but that trend is likely to continue only if international support for a university's programmes is maintained in all but the most extraordinary circumstances, such as those now existing in China, where such support requires a degree of cooperation with the home government that is squarely inconsistent with the home government's actions against university welfare. Even in the case of China, international support should be eagerly reinstated as soon as conditions become acceptable, because, as with South Africa, the ability of the nation to make a transition toward a more open society depends, in part, on the vitality of the university. The university, much like the church, should be boycotted only when the institution itself cannot serve its universal mission under government stricture. A foreign government's displeasure can, in most situations, be conveyed by less injurious means.

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Journal costs

SIR—My mail this morning contained no fewer than half-a-dozen announcements of journals to which my poverty-stricken library was invited to subscribe. In every single case, the subscription costs quoted included both an 'individual rate', and an 'institutional rate' of anything up to twice as much. As medical staff in this country have received pay awards at least in line with official inflation figures, whereas my library budget has remained the same for the past three years, this seems unfair and ultimately self-defeating.

The problem lies in the fact that the production of new journals is, on the whole, supply-led rather than demand-led. Scientists are not so eager to learn of new developments in their specialized sub-fields as to be willing to put their hands in their pockets and pay for the knowledge contained in new journals. Rather, it seems to be the case that scientists are well aware that appointment, tenure, promotion and grant-awarding go by the number of papers published, and so are keen to find new outlets to publish in. I have heard rumours of one who boasted of having managed to publish 16 papers based on the same set of data. Although this is, I hope, an extreme case, it is undoubtedly common to find that several parts of what is clearly the same study have been published, under different permutations of authors' names, in different journals, thus making it impossible for an individual with a personal subscription to one relevant journal to cover the study adequately.

The solution seems to me to be to find some other way of selecting candidates for appointment, tenure and so on. Surely the best and brightest minds of the scientific world, currently fully engaged in making sure that their names are somewhere in the list of authors of anything their graduate students publish, could find some better method of assessing each other? If the only scientific papers published were those needed to inform other scientists of new developments, a considerable proportion of these new journals would die of inanition.

If your own publication, Sir, was genuinely devoted to maximizing the distribution of scientific information, I am sure that you would admit that handling institutional subscriptions is less work for you than handling individual subscriptions, and would be offering institutional subscriptions at half the price of those of individuals, rather than the other way round.

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■ See also p.349.

Making good databanks better

Like motherhood before Green considerations made it unfashionable, the scientific community's databanks enjoy the presumption of virtue. But there remain important problems to be solved.

It is splendid, and entirely consonant with the doctrine that the scientific enterprise is a communal enterprise, that data arising in the course of discovery should be generally available. Access is especially important when a claim can be checked only by reference to the original data, but it must often be that data gathered from several investigators is more valuable in a common databank than if separate parcels remain where they arise.

So why does *Nature* not make it a precondition of publication that experimental data should be submitted to the appropriate databank? The question is more often asked as databanks proliferate, and as compliance with the pleadings of the databanks becomes more common. What follows is an explanation both of this journal's continued caution and of why that diffidence does not imply dissent from the objectives of the databanks.

The sheer number of databanks now extant is part of any explanation. The days have gone when the Crystallographic Data Centre at the University of Cambridge, created in the 1930s by the International Union of Crystallography, was the chief organization of its kind, but that has been the model for several more recent creations, notably the nucleotide sequence databanks operated in concert at the European Molecular Biology Laboratory (EMBL), Heidelberg, the Los Alamos National Laboratory and at the Riken Laboratory at Tokyo in Japan. Now, largely on the initiative of Dr Fred Roberts at Yale University, an international network (centred on the Brookhaven National Laboratory) is also being formed to collect three-dimensional structure data on proteins and other structures.

Such databanks function on common principles. The submission of data is voluntary, but access is general. Organization is often a formidable undertaking, as the nucleotide sequence databanks have discovered in the past five years. Compilations of data are available to all, usually at the modest cost of the magnetic tape or compact disk that carries them. They are a considerable public service.

Other databanks function differently. Part of the legacy of international projects such as the International Geophysical Year is the network of data repositories scattered about the globe, while particular Earth satellites have led to the creation of their own data repositories whose value persists — data from the Einstein X-ray

satellite, launched more than a decade ago, are still being used, for example (see page 309, this issue). Cores drilled in the ocean floor are similarly available, while it is intended that data collected by the Hubble Space Telescope will be open to all once the principal investigators have had a crack at them. These are sensible ways of making information generally available, but because these databanks are created by the voluntary acts of single projects, they pose no difficulties for researchers or for journals.

Problems of principle do arise when success rests on the willingness of individual researchers to submit their data. This is the case with the internationally integrated nucleotide sequence databank — but nothing in what follows implies that the objectives of that enterprise are anything but excellent.

One difficulty is geographical. As things are, the three collaborating centres have divided the world between them, preferably taking in data electronically from individual researchers, but otherwise undertaking to type in manually data appearing in journals. Access to this databank will soon be possible by computer. One obvious difficulty is that access is patchy. People in, say, India have less easy access than those elsewhere, and may feel doubly injured by demands that the contribution of their data to the common pool from which others will benefit most should be a precondition of publication.

The Soviet Union is in worse shape; the Soviet Academy of Sciences buys an updating tape from Heidelberg each month, but has been told that it cannot be a full partner in the collaboration for many different reasons — that full membership would entail information about the operation of computers covered by the strategic embargo, that three partners are a sufficient headache for the time being and that, in any case, the Soviet Union does not produce many nucleotide sequences. Even allowing that the task of accumulating sequences is herculean, an enterprise that seeks to be international and comprehensive could have spent more time worrying about equality of access.

Commercial difficulties also arise. Academic researchers may regard submission as the rule, but those working for commercial organizations will be more restrained. Sequences published *in extenso* will be available, but what about the rest? Commercial organizations are

notoriously unwilling to let their competitors know what interests them, and may legitimately claim the right to secrecy while enjoying free access to what academics publish. That is why the databanks have been reduced to pleading with commercial companies at least to keep their sequences securely.

Still more worrying is the commercial use of nucleotide sequence data. Already there are several small companies selling packages of proprietary interpretive software together with the contents of the databanks. It is not difficult to think that there will soon be consultancy firms offering to interpret the contents of the databanks for the benefit of commercial clients. The development of such services shows a need for them, but their equitability as it affects the providers of the data and even the databanks themselves has been inadequately explored.

The three-dimensional structure databank raises another difficulty. The case for it is strong: the publication of a molecule's structure may be useless to others without more detail, atomic coordinates for example. But it may be easier (and quicker) to reach broad conclusions than to refine the atomic coordinates, making them unambiguous. So authors will enjoy a moratorium between publication and the submission of data that will no doubt also ensure that no "sharpshooting theorist" (one researcher's phrase) is the first to interpret the structure.

The moral for journals such as this is plain. Their first duty is to speed the process of publication but also to ensure (as far as possible) the integrity of what they publish, which gives them a right to ask for supporting data (sequences, atomic coordinates) even when they have no space to publish them. They should do that more often, and should also arrange to provide access to them. But journals have no right to adjudicate upon a contributor's subsequent conduct — and have few sanctions, anyway. If there must be policemen, grant-making agencies are better placed. But there is a prior need — that the unanswered questions of access and equity should be tackled energetically by some body such as the International Council of Scientific Unions. Meanwhile, *Nature* will continue to urge on its contributors the importance of submitting their data to the databanks, but will not exact unenforceable promises to do so.

John Maddox

Mineralogical phase change at the 670-km discontinuity

Bernard J. Wood

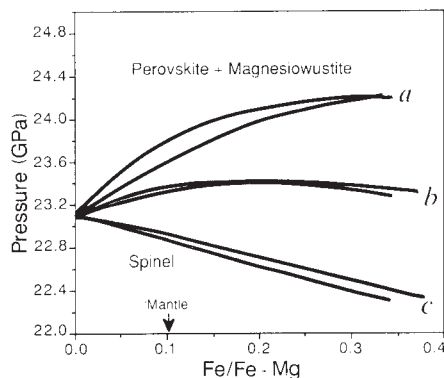
THE boundary between the upper and lower portions of the Earth's mantle is generally taken to correspond to the worldwide seismic discontinuity at 670 km depth, at which the seismic-wave velocity increases by a few per cent¹ and the density of the silicate minerals increases. The simplest explanation, that the discontinuity is due to an isochemical phase transformation in a mantle of uniform composition, is disputed in the light of incompatible experimental data² on the thermodynamic and elastic properties of high pressure phases. An alternative model is that the mantle is chemically stratified with at least one major compositional discontinuity at 670 km and possibly other distinct layers at shallower depths. But in a new experimental study⁴, Ito and Takahashi show that an isochemical transformation in $(\text{Mg,Fe})_2\text{SiO}_4$, the dominant component of the upper mantle, has many of the properties required to explain the 670-km discontinuity. Their results would thus be consistent with both upper and lower parts of the mantle being peridotitic in composition.

The question of whether the mantle, which is convecting vigorously, is chemically layered is crucial to understanding its dynamics and its chemical and physical history. Numerical simulations⁵ indicate that even small compositional differences, equivalent to a 3-per-cent change in density, would be sufficient to inhibit convective exchange between the upper and lower mantle and hence to isolate the lower mantle from most plate-tectonic processes. Many geochemists have argued that such isolation occurs, on the grounds that it explains the apparent depletion of the source region of oceanic basalts in lithophile elements such as K, Rb, Cs, U and Th relative to the inferred composition of the bulk Earth. Modelling⁶ of the isotopic evolution of this source region implies that it corresponds to about 30 per cent of the volume of the mantle, which happens to equal the volume of the mantle above 670 km.

Seismologically, the 670-km discontinuity is extremely sharp, giving rise to underside primary-wave reflections that require the velocity increase to take place over a depth interval of less than 4 km. Isochemical transformations in complex systems are generally continuous and spread out, however, implying that they cannot, in general, give rise to reflections at the wavelengths of concern. Chemical stratification, on the other hand, would give a true discontinuity.

The experiments of Ito and Takahashi⁴

relate to the conditions under which $(\text{Mg,Fe})_2\text{SiO}_4$ in the spinel structure transforms isochemically to $(\text{Mg,Fe})\text{SiO}_3$ perovskite plus $(\text{Mg,Fe})\text{O}$ magnesio-wüstite at high pressures. The authors show that the transformation is extremely sharp for compositions with upper-mantle ratios of $\text{Fe}^{2+}/(\text{Fe}^{2+} + \text{Mg})$ of about 0.1 and that it takes place under pressure conditions—23 gigapascal (GPa) at 1,600 °C—that closely approximate those present at the 670-km discontinuity. For these low iron contents, the experimental data indicate that there exists a narrow transition interval of coexistence of high- and



The breakdown of $(\text{Mg,Fe})_2\text{SiO}_4$ spinel to perovskite and magnesio-wüstite at 1,600 °C (after ref. 4). Note that the transition interval of coexistence of all three phases has a maximum width of 0.2 GPa (6 km) if we use possible geometry *a*. The most likely geometries, however, are *b* or *c* which give extremely narrow transition intervals for upper-mantle values of $\text{Fe}^{2+}/(\text{Fe}^{2+} + \text{Mg})$.

low-pressure phases and that the transformation pressure may either increase slightly or decrease as Fe is substituted for Mg (see figure). But even if we assume large uncertainties in the partitioning of Fe and Mg between low- and high-pressure phases, it is virtually impossible to generate an interval wider than 0.2 GPa (6-km depth interval) and values less than 0.05 GPa (1.5 km) are most likely. This leads to the unexpected conclusion that the isochemical transformation of $(\text{Mg,Fe})_2\text{SiO}_4$ spinel to perovskite plus oxide is essentially discontinuous and is thus capable of acting as a seismic-wave reflector.

The phase transformation may exhibit one of the main features of the 670-km discontinuity, but is it consistent with other geophysical and geochemical data? Jeanloz and Knittle argue in another new paper⁷ that the density of the lower mantle (known to an accuracy of about 0.5 per cent) is too high for it to be compositionally equivalent to upper mantle

peridotite. From their measurements of the compressibility and thermal expansion of the perovskite phase, they infer that the lower mantle is enriched in iron relative to the upper mantle and hence, by implication, that the 670-km discontinuity corresponds to both a chemical boundary and a phase transformation. This argument depends, however, on measurements of the thermal expansion of perovskite at low pressure, well outside the perovskite stability field², that gave a high coefficient of thermal expansion. There is doubt about the applicability of these data, collected on a metastable phase and requiring much extrapolation in pressure and temperature, because measurements of other perovskite-like phases⁸ yield lower thermal expansions. Adoption of a smaller thermal expansion coefficient brings the density of lower mantle into approximate agreement with that expected for peridotite compositions (C.R. Bina and P.G. Silver, personal communication).

Although geochemical data clearly show long-term segregation of the mantle into regions distinct in isotope and trace-element evolution, there is no requirement that these reservoirs exhibit gross differences in major-element composition or that they be radially distributed above and below 670 km depth. Furthermore, there is increasing seismological evidence that, at plate boundaries, subducting slabs penetrate into the lower mantle¹⁰, implying that there must be at least limited chemical interchange across the 670-km discontinuity. Although chemical stratification at 670 km provides a convenient explanation for the isotope data, mixing of the upper and lower mantle would also be inhibited, but not completely stopped, by a phase transition with a slightly negative pressure-temperature dependence. The transformation studied by Ito and Takahashi fulfils this requirement. Thus the new experimental data indicate that a simple phase change explains the discontinuity. Measurements of the density of perovskite plus magnesio-wüstite under actual lower-mantle conditions are required to refute or confirm this. □

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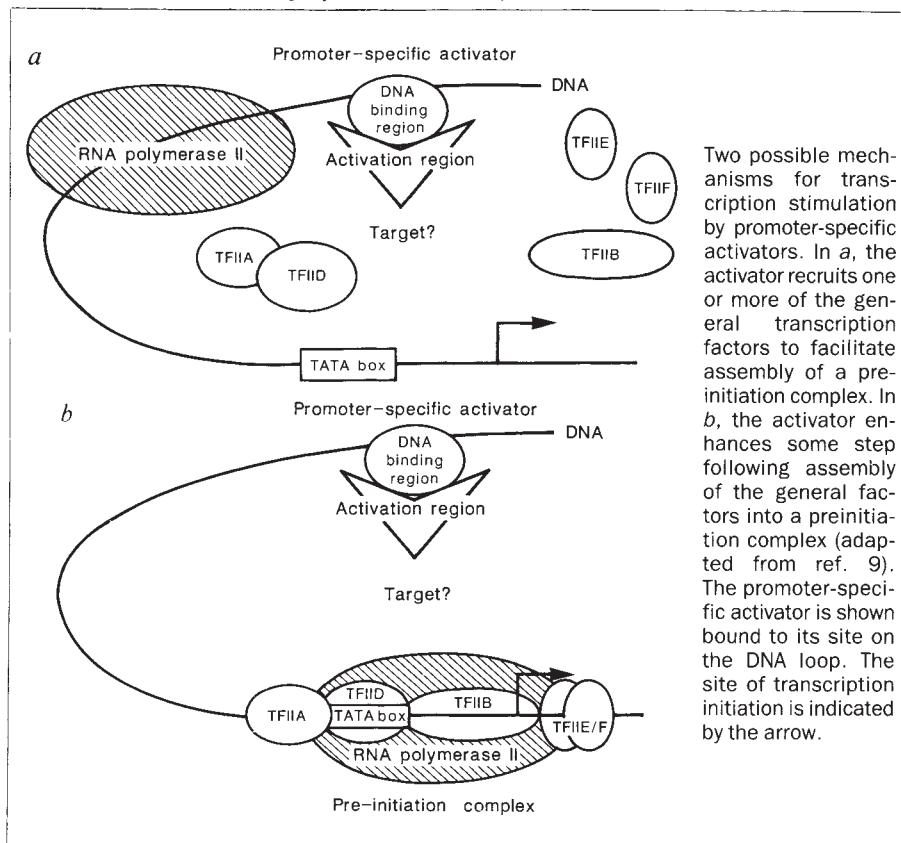
Activator's target in sight

James W. Lillie and Michael R. Green

ALTHOUGH accurate transcription of a eukaryotic protein-coding gene was first reconstituted in a cellular extract ten years ago^{1,2}, we do not yet know what the components of these extracts are nor how they are regulated. In the interim, we have stockpiled the genes of many promoter-specific activators, which augment the transcription of particular genes, and several subunits of RNA polymerase II,

polypeptides are normally associated with one another in the cell. Have natural complexes been disrupted by chromatography and do other, unrelated proteins remain unseparated?

Progress has been slowed by the difficulty in obtaining a pure, concentrated supply of each factor. The purification of mammalian transcription factors is expensive and arduous. To make matters



Two possible mechanisms for transcription stimulation by promoter-specific activators. In *a*, the activator recruits one or more of the general transcription factors to facilitate assembly of a pre-initiation complex. In *b*, the activator enhances some step following assembly of the general factors into a preinitiation complex (adapted from ref. 9). The promoter-specific activator is shown bound to its site on the DNA loop. The site of transcription initiation is indicated by the arrow.

the enzyme that transcribes all protein-coding genes. But most basic components of the transcription machinery remain elusive. It is therefore exciting that two genes encoding basic transcription factors have been cloned as reported in refs 3–6 and on page 299 of this issue⁷, and that the cloning of other general components seems at hand. Once these basic factors are identified, we may better ask how transcription is regulated.

Crude extracts of mammalian cells have been broken into at least five fractions (originally TFIIA through TFIIE) that are required by RNA polymerase II for the accurate transcription of a DNA template (reviewed in ref. 8). As TFIIC has become a relic and the original TFIIE has been split into IIE and IIF, we are left with TFIIA, IIB, IID, IIE and IIF. But what are these factors and how do they work? We do not know how many polypeptides comprise each factor nor which of the

worse, the least abundant and most labile factor — TFIID — is required for the first step leading to transcription initiation (see ref. 9). Without TFIID, no transcription event can be studied. For this reason, the discovery that yeast contain a readily purified substitute for the mammalian TFIID fraction was a major breakthrough^{10–12}. It then became feasible to sequence a piece of the purified protein and construct a probe to clone the gene^{4,5,7}. Meanwhile, the *in vivo* experiments required to authenticate the cloned gene had been completed independently. Yeast geneticists, also working to identify genes encoding basic transcription factors, simultaneously identified this locus as one important for regulating transcription³.

In a separate development, biochemists working to identify proteins that stick to RNA polymerase II, cloned a gene encoding another basic factor, RAP30,

a subunit of the TFIIF fraction⁶. RAP30 comes bound tightly to another polypeptide, forming a heteromeric complex called RAP30/74 (ref. 13). This complex is required for the final stages of transcription initiation and for chain elongation. Besides binding tightly to RNA polymerase II, RAP30/74 is an ATP-dependent DNA helicase⁶.

Sigma homologue?

It is the implicit hope of all gene cloners that the amino-acid sequence deduced from the gene sequence will provide a clue to how the protein works. The cloners of both TFIID and RAP30 claim their protein is similar in sequence to bacterial sigma factor^{6,7}. Sigma, the most loosely associated component of bacterial RNA polymerase, is required for accurate initiation of transcription. There has been much speculation that one of the eukaryotic transcription factors is the sigma homologue. Although TFIID and RAP30 cannot both be the sigma homologue, perhaps each represents one portion of sigma.

The diverse activities of sigma, which include RNA polymerase binding, promoter recognition and DNA melting (see ref. 14) may be distributed among several eukaryotic factors. If so, it would seem reasonable that TFIID, which recognizes the TATA box, an AT-rich sequence upstream of the start site of transcription, has homology to a sigma region that is thought to contact the TATA-like sequence (–10) of prokaryotic promoters⁷. And it would not be surprising if RAP30, which binds to RNA polymerase II, is similar to a sigma region believed to bind bacterial RNA polymerase⁶. To extend the analogy, we may find that RAP74, the other subunit of TFIIF and a probable ATP-dependent helicase, is similar to the region of sigma posited to melt the DNA base pairs spanning the transcription initiation site⁶. In this light, we note a precedent for a fragmented sigma factor: a bacteriophage SPO1 sigma factor is composed of two proteins (see ref. 14).

Multiple TFIIDs?

Prokaryotes have multiple sigma factors, and different sigma factors direct RNA polymerase to different promoters¹⁴; do eukaryotes have more than one TFIID? Among eukaryotic promoters there is a wide spectrum of TATA-box sequences, and some promoters appear entirely to lack a TATA box. But as purified TFIID, from either mammalian cells^{15,16} or yeast¹⁷, binds to and acts upon all promoters tested, the existence of different TATA sequences may not necessitate multiple TFIID factors. Similarly, the widespread notion of 'TATA-less' promoters may be incorrect. At the very least, the cloned TFIID is the predominant TATA box

binding protein of yeast: all laboratories have cloned the same yeast gene and none has detected a related sequence or TATA-binding activity.

There are, however, two promoters that may have no requirement for TFIID: the yeast *HIS4* T₁₈ and the mammalian U2 snRNA¹⁹ promoters. These two promoters lack a canonical TATA box, and, in contrast to most genes, they are neither activated nor repressed by typical promoter-specific activators. Perhaps these promoters lack the target of the activator. Is the target TFIID?

Promoter-specific activators

Transcription by basic transcription factors is augmented by promoter-specific activators. These bind to short specific sequences within the vicinity of the gene. Genes encoding yeast activators have been cloned by classical genetics; but the strategies used to clone the genes encoding mammalian activators take advantage of the activator's affinity for a specific DNA sequence. In addition to the DNA-binding domain, activators contain an 'activating region', which is required for transcriptional activity (reviewed in ref. 20). Its name is testimony to how little we know of its structure or function. The sequence of an activator is of little help in deducing the position of the activating region; so far, activating regions are characterized primarily by a preponderance of a particular amino acid (for example, glutamine or proline, or acidic amino acids in general; see refs 20 and 21).

Activators enhance one or more steps leading to transcription initiation or elongation. Presumably this is due to direct interaction between the activating regions of these proteins and some component of the basic transcription machinery. General transcription factors interact with the promoter in a step-wise fashion to form a preinitiation complex (a cartoon of which is shown in the figure). In principle, any rate-limiting step in this assembly could be regulated by activators. One potential rate-limiting step is binding of TFIID (or a complex of proteins including TFIID) to the TATA box, the first step in complex formation.

Several lines of evidence suggest that TFIID is the target of the activator. A viral activator, the pseudorabies virus immediate-early protein, facilitates TFIID-promoter interactions: preincubation of the DNA template with purified TFIID obviates the need for the viral activator²². Also, TFIID affects the off-rate of a cellular activator, USF²³. In addition, two cellular activators, mammalian ATF and yeast GAL4, alter the DNase I footprint of partially purified mammalian TFIID and increase protection over the TATA box^{24,25}. Although these experiments are suggestive, there is no direct evidence linking the interaction of an

activating region and TFIID to transcription activation.

Interactions between highly purified yeast TFIID and the activator Sp1 have not been detected²⁶. This does not necessarily contradict the GAL4 and ATF studies, as the target of the Sp1's glutamine-rich activating region may be different from that of GAL4 and ATF. Comparison of these studies is further complicated by the different preparations of TFIID used. The purified yeast TATA-binding activity of the Sp1 experiments has *M_r* of 27,000 (27K). The less purified mammalian TFIID used in the GAL4 and ATF experiments seems to be much larger²⁷ and protects a more extensive promoter region¹⁵ than the yeast 27K protein¹⁰. Perhaps the 27K protein is only one component of a larger complex, which may be the actual target of the activator. Such a complex could be disrupted during purification and then reassembled when the purified 27K protein is added back to the other relatively impure fractions. In fact, there is evidence that TFIID interacts with TFIIA⁹. Not all investigators require or use a separate TFIIA fraction^{24,25}, perhaps because the TFIIA polypeptide(s) remain associated with the TATA-binding activity.

Multiple targets?

While much effort has been expended in detecting interactions between gene-specific factors and TFIID, other possibilities remain to be explored. Given the apparent diversity of activating regions²¹, it would be surprising if they all worked identically. There is evidence that some activators interact with RNA polymerase II, in particular its tail — the phosphorylated carboxyl-terminal repeat structure of the largest subunit. Removal of the polymerase tail eliminates transcription^{28,29}, and the effects of partial tail deletion can be compensated by a strong activator³⁰. In addition, a column containing the activator GCN4 retains RNA polymerase II³¹. Furthermore, some activators may recruit basic transcription factors to the promoter, whereas others may function only after all the basic factors have been assembled (see figure); prokaryotic activators, for example, either recruit RNA polymerase to the promoter or act after RNA polymerase has bound (see ref. 32).

Other eukaryotic activators are implicated in regulating the elongation of the RNA transcript. The uninduced *hsp70* gene of *Drosophila* has a transcriptionally engaged RNA polymerase that forms a 25-nucleotide nascent RNA. RNA polymerase II is arrested at this position in the absence of heat induction³³. And the transcription of *c-myc* may be down-regulated by retinoic acid in HL60 cells through premature termination³⁴.

Clearly there is still a great deal to be learned. But the cloning of TFIID and

RAP30 provides two more important reagents to understand eukaryotic transcription regulation. Ultimately, the reconstitution of transcription from purified components will be required to understand how activators interact with basic transcription factors. This may happen sooner than originally anticipated. The other subunit of TFIIF, RAP74, should soon fall to the cloners. More RNA polymerase II subunits are in the process of being cloned from yeast. The recent finding that yeast contains an equivalent to TFIIA (ref. 35) will undoubtedly facilitate cloning of the TFIIA gene. Finally, the power of yeast genetics will lead to the cloning of other genes. Understanding the exact assembly of the transcription complex and the role of each factor is a target in sight. □

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ASTRONOMY

Faint active galaxy puzzle

Lance Miller

ALMOST by definition, one would expect an active galactic nucleus to be bright. Yet A. V. Filippenko and W. L. W. Sargent report (*Astrophys. J.* **342**, L11–L14; 1989) that they have identified such a nucleus that is exceptionally dim. They recognized the active nucleus by its spectral features, but note that its optical luminosity is 10^3 times less than is usually associated with its type — indeed, it is less luminous than the brightest supergiant stars in our own Galaxy. Furthermore, the host galaxy is very unusual — a nearby dwarf that is a factor of about 10 less luminous than the galaxies in which active nuclei are usually found. The new results, thus, seem to follow in a frustrating tradition in research on active galaxies, whereby important new data confuse rather than clarify the issue.

Active galactic nuclei are extraordinarily bright regions at the heart of host galaxies, and are often termed Seyfert galaxies. Most commonly, it is supposed that their great luminosity is generated by the vigorous heating of gas and dust being accreted onto an extremely massive object, probably a black hole in their centre (see the recent News and Views article by Cedric Lacey, *Nature* **340**, 675–676; 1989). The new active nucleus appears as a faint star-like knot close to the centre of the galaxy NGC4395. Previously, it was assumed to be an H II region, a site of active star formation, although it was recognized as being extremely unusual (M. L. McCall, P. M. Rybski & G. A. Shields *Astrophys. J. Suppl.* **57**, 1–62; 1985).

Filippenko and Sargent have now obtained high-quality spectra of the knot, and these have the main characteristics of more luminous active nuclei; they identify the object as having the spectral features of a type-1.8 Seyfert nucleus. The profiles of the spectral lines due to hydrogen Balmer emission have strong narrow cores, indicating that the internal motion of the gas is largely negligible, but faint broad Doppler wings indicate the presence of some gas moving as fast as $3,500 \text{ km s}^{-1}$. Other emission lines indicate that the gas is present in a wide range of ionization states, for example both Fe^{2+} and neutral oxygen being detected. Filippenko and Sargent argue that the ionization is caused by a continuum of high-energy radiation, not by shock heating of the gas which would give different emission-line intensities.

The optical continuum underlying the atomic line emission is featureless, there being no absorption lines attributable to the presence of late-type stars. The relative strength of the emission lines and the continuum is in the range expected for

Seyfert nuclei. Nevertheless, the spectrum does have some unusual features: despite the broad wings on 'permitted' lines, such as the Balmer lines, lines due to 'forbidden' transitions are extremely narrow, corresponding to a spread in atomic velocities less than 60 km s^{-1} , considerably below the value usually found for Seyfert galaxies, 300 km s^{-1} ; and it seems from the line spectrum that the abundance of nitrogen relative to that of oxygen is anomalously low.

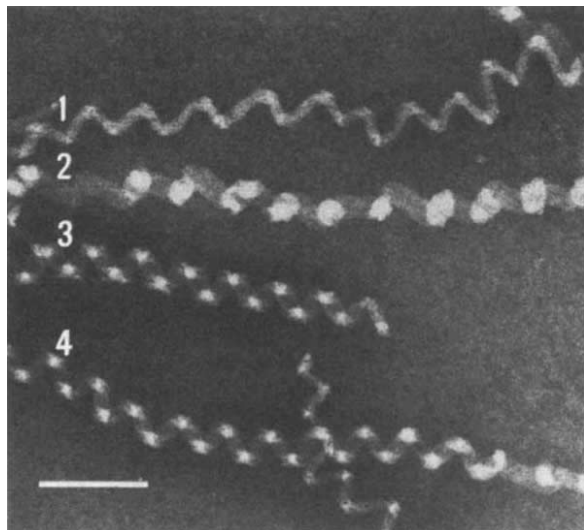
The principal puzzle concerns the source of the ionizing radiation. Because the spectrum looks like that from a normal active nucleus, it is reasonable to suppose that accretion onto a massive object is

responsible. If this turns out to be the case, it would highlight how little we know of the origin of the activity. The brightest active galaxies include extended, powerful radio sources and are all massive elliptical galaxies. One can infer that the central object in these sources must contain at least 10^6 solar masses, as this is the minimum amount of material that must have been accreted to account for the energy stored in the radio lobes. Lower-mass, radio-quiet active nuclei are found mostly in lower-mass spiral galaxies, but even these are 10 times as massive as the dwarf galaxy containing the nucleus studied by authors. This most basic of observational trends relating the host galaxy to the active nucleus is not understood at all.

The authors put forward an alternative (hypothetical) source of the ionizing radiation, which would also account for

Self-assembling lipid helices

HELIX formation, it seems, is not the exclusive privilege of biopolymers. For the helical structures shown in these electron micrographs are formed not by continuous strands of nucleic acids folding to form internal networks of hydrogen bonds, but by the spontaneous self-assembly of chemically modified phospholipids, the principal constituents of biological membranes. In aqueous media, natural phospholipids have a tendency to aggregate forming micelles, monolayers and bilayer vesicles. But Hiroshi Yanagawa and co-workers at the Mitsubishi Kasei Institute of Life Sciences in Tokyo have discovered that simply by attaching the nucleoside cytosine, which contains one of the four bases responsible for helix formation in DNA, to the hydrophilic ends of phospholipids, they endow the molecules with the ability to form helical rather than layered structures.



Although the precise arrangement and degree of ordering of the modified phospholipids within the helices is not yet clear, it seems likely that the helical nature of the aggregates is a consequence of stacking and hydrogen bonding between nucleoside bases. Following their first report (Yanagawa, H., Ogawa, Y., Furuta, H., Tsuno, K. *Chem. Lett.* 269; 1988) describing the circular and linear helical strands formed by the phospholipid–nucleoside conjugates dipalmitoyl-5′-phosphatidyl cytosine and dipalmitoyl-5′-phosphatidyldeoxycytidine, Yanagawa and colleagues tested the effect on helix formation of shortening the alkyl chains of the lipid. They found that in contrast to the modified phospholipids of longer chain length, which form simple helices, dimyristoyl-5′-phosphatidyldeoxycytidine forms superhelices — helices of helical strands — examples of which are shown above. Strand 1 is a right-handed duplex with a diameter of about 110 Å and helical pitch of about 240 Å whereas strands 2–4 consist of a double duplex with similar diameter and helical pitch. The superhelical pitches of each of these strands are about $1,000 \text{ Å}$.

Commenting on these findings in a recent issue of the *Journal of the American Chemical Society* (111, 4567–4570; 1989), the authors propose a structural model for the single-stranded helix in which the conjugates are arranged in the form of a twisted micellar lipid bilayer — a kind of molecular spiral staircase in which the nucleoside groups frustrate the natural tendency of the lipids to stack side-by-side. David Concar

the low optical luminosity — a 'warmer', which is a cluster of highly evolved, hot, massive stars. R. Terlevich and J. Melnick suggested (*Mon. Not. R. astr. Soc.* **213**, 841–856; 1985) that such an object, in which stars could have effective temperatures as high as 100,000 K, could easily be mistaken for a Seyfert nucleus, having the same characteristics of ionizing radiation. The positive identification of one warmer would greatly support the hypothesis that many other galaxies have mistakenly been identified as Seyferts. The biggest problems in applying the warmer model to the nucleus of NGC4395 are accounting for the high velocities seen in the permitted lines, which imply extremely energetic outflow, for which no mechanism

seems to be available, and the lack of spectral features seen in Wolf-Rayet stars, thought to be akin to the stars in a warmer.

Finally, if the nucleus studied by Filippenko and Sargent is in the family of accretion-driven Seyfert galaxies, it implies that even low-mass galaxies can be hosts to active nuclei, and that such faint nuclei may be common and are unknown because they are apparent in only the nearest dwarf galaxies. It is hoped that further studies of the nuclei of similar galaxies will clarify the relationship between galaxy type and activity. □

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DROSOPHILA DEVELOPMENT

Making stripes inelegantly

Michael Akam

A STRIKING feature of *Drosophila* embryogenesis is the early appearance along the antero-posterior (A-P) axis of a precisely repeating pattern, the stripes of expression of the pair-rule genes^{1,2} (see figure). This pattern depends upon the earlier expression of the segment gap genes, but these do not provide periodic cues; different gap genes are expressed in zones of varying width along the axis of the embryo. Periodicity might be generated in one of two ways. An elegant mechanism, favoured by model builders^{3,4}, would use an intrinsically periodic pattern-

interaction with a gradient of the protein expressed from the *bicoid* gene; successively more posterior regions of the embryo express *Krüppel* (*Kr*), then *knirps* (*kni*)^{2,9}. When the distribution of transcripts from these genes is analysed, the region expressing each appears to be sharply bounded, defining abutting¹⁰ or slightly overlapping¹¹ zones of expression. But the protein products of the gap genes are not so precisely localized. Antibodies reveal that the hunchback and Krüppel proteins accumulate in broader domains with no well-defined boundaries (see

Fig. 4 of Stanjovec *et al.* on page 334 of this issue⁸). The apparent limits of these domains depend considerably on the sensitivity of the antibody staining techniques used¹¹. Even so, it is clear that they overlap substantially. Within these regions

of overlap, the relative concentrations of two gap proteins change continuously over a distance of at least 8–10 nuclei (about 2–3 segment primordia). It seems very likely that these graded changes in concentration and/or ratios of the gap gene products are providing cues for subsequent pattern generation.

Genetic studies indicate that all of the pair-rule genes are dependent on the gap-proteins for normal patterning, but that only three 'primary' pair-rule genes, *hairy*, *runt* and *even-skipped* (*eve*), are absolutely required to generate the periodic pattern⁷. Two of these primary pair-rule genes have now been shown to contain discrete promoter elements that mediate the generation of different parts of the stripe pattern. The first hint of this

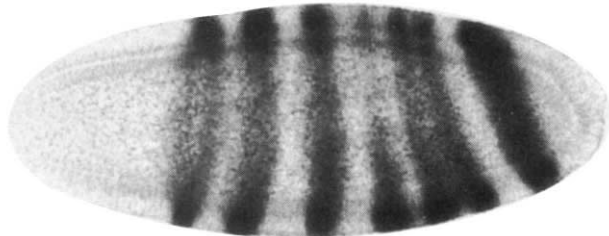
came from the study of mutations at the *hairy* locus⁵. Whereas most *hairy* mutants display segment fusions throughout the whole embryo, a subset of mutants causes segment fusion only in particular regions of the embryo. These mutants turned out to have deletions of different parts of the 5'-regulatory region of *hairy*. This implied the presence of region-specific promoter elements, in marked contrast to the organization of the promoter for the 'secondary' pair-rule gene *fushi-*tarazu** (*ftz*)¹². At *ftz*, promoter deletions generate either the complete set of stripes, or no stripes. This different organization supports the view that *hairy* responds directly to signals from the gap genes, whereas *ftz* responds only to periodic signals, generated by the pair-rule genes themselves.

A series of papers dissecting the regulation of the *eve* locus have amplified and confirmed this model^{6,8,13}. The *eve* promoter proves to be particularly complex, in that it has both gap-response elements that mediate its initial activation, and a functionally independent element that responds to the accumulating products of the pair-rule genes. The pair-rule element becomes active somewhat later than the gap-response elements, and directs a distinct pattern of slightly narrower stripes.

So far, the gap-response elements have only been defined by deletion: regions of 1–2 kilobases of DNA within the 5'-regulatory region of *eve* specifically activate either stripe 3 or stripes 2 and 7 of the *eve* expression pattern. When truncated promoters containing the gap-response elements, but not the pair-rule response element, are re-inserted into embryos, they mimic very precisely the early expression of the endogenous *eve* gene in stripes 2, 3 and 7 (ref. 6). This activity remains dependent on the gap-gene products, but is unaffected by mutations in the pair-rule genes, implying that input from the gap genes alone can specify the location and width of the initial *eve* stripes.

It has been postulated for some time that the three gap genes, *hb*, *Kr* and *kni*, may be acting directly as transcription factors. Each encodes a putative zinc-finger protein^{14–16} consistent with that notion. Strong evidence for this view is provided by three papers in this issue^{8,17,18}. These demonstrate that the hunchback and Krüppel proteins bind to specific DNA targets. One of these studies examines the binding of these gap proteins to the *eve* promoter. Using footprinting techniques, Stanjovec *et al.*⁸ find multiple binding sites for both proteins. Most of these are clustered within the elements that direct specific stripe formation.

The two other papers examine binding of the gap proteins to different potential target sequences. Treisman and Desplan¹⁷ identify hunchback and Krüppel binding



Stripes on a *Drosophila* embryo — in this case somewhat abnormal *ftz* stripes (from ref. 22).

generating system, comprising the pair-rule genes and their products. This would only need to be triggered by local stimuli from the gap genes. Alternatively, unique instructions could be generated by the gap-gene proteins to define the position of each pair-rule stripe. Analysis of the interaction between gap and pair-rule genes seems to favour the less elegant 'specific instruction' process. Discrete elements within promoters for the pair-rule genes contain specific targets for the binding of one or more gap-gene proteins. These elements direct the expression of single stripes along the A-P axis⁸.

The expression of three gap genes spans the greater part of the segment pattern. The *hunchback* (*hb*) gene is activated in the anterior half of the embryo by direct

sites upstream of the *hb* promoter; Pankratz *et al.*¹⁸ define Krüppel binding sites upstream of the *kni* promoter. In each case, a very similar DNA sequence characterizes the Krüppel binding site, although *Kr* has been implicated as a negative regulator of *hb*, but a positive regulator of *kni*. The proposed hunchback binding sequences are more divergent. (It should be noted that there is no evidence that the hunchback protein binds to its own promoter, so the sequences defined by Treisman and Desplan¹⁷ need not be functional.)

If Krüppel is acting directly by binding to the promoters of *kni*, and of the pair-rule genes, as these results suggest, then it must be functional at concentrations where the protein is barely detectable by present techniques. Pankratz *et al.* point out that an effect of Krüppel is observed throughout the zone of *kni* transcription, even though part of this lies beyond the detectable limits of Krüppel protein. *Kr* mutants alter the pattern of *eve* transcription in a region spanning *eve* stripes 2–6 (ref. 13), again suggesting that Krüppel is effective at, or beyond, the apparent limits of its distribution. This evidence for the action of the gap proteins at low concentrations throughout relatively large numbers of segment primordia, raises the intriguing possibility that the gap genes are effectively generating secondary gradients within the egg, extending from the boundaries of their zones of transcription.

The gap genes have been implicated in the primary regulation of the homoeotic genes, as well as in the segmentation hierarchy^{1,19,20}. If the gap proteins are functional over a wide concentration range, they could provide positional information for the definition of precise segment identities, as well as for the positioning of the pair-rule stripes. Such

information must be available within the blastoderm, but the discrete domains of gap-gene expression do not appear to provide sufficient resolution for this purpose. Local gradients of gap protein concentration could, however, provide the needed resolution.

If each pair-rule stripe is generated by the interpretation of a unique instruction, then the apparent simplicity of the repeating segment pattern is deceptive. A 'specific instruction' mechanism could generate an arbitrarily complex set of uneven stripes. The simplicity of the natural pattern must then be attributed to a process of selection needed to produce a

set of evenly sized segment primordia.

Where does this leave spontaneous pattern-generating mechanisms? One very likely role for these processes is to amplify the initial discontinuities in pair-rule gene expression, and to sharpen the boundaries between stripes²¹. In this respect, the feedback circuits of the pair-rule genes act as bistable, or perhaps multi-stable, switches, forcing cells into one of a few preferred states. But it seems that they do not define periodicity. □

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ATMOSPHERIC OZONE

Ultraviolet levels down not up

Stuart A. Penkett

Most people are familiar with the danger of ozone depletion in the upper atmosphere, and its probable consequences. Fewer are aware that the concentration of ozone in the lower atmosphere may be increasing. According to C. Brühl and P. J. Crutzen, in a recent issue of *Geophysical Research Letters* (16, 703–706; 1989), this increase could counteract the effect of the stratospheric depletion on the amount of ultraviolet radiation reaching the Earth's surface, especially in the Northern Hemisphere. This could mean that the increased incidence of skin cancer that many fear will follow stratospheric depletion, may not materialize. Instead, the effect in the changing concentrations would be seen most in atmospheric temperatures, with a warming of the troposphere and a cooling of the stratosphere.

Interest in the behaviour of tropospheric ozone is growing strongly, because it is an efficient greenhouse gas, it probably causes damage to vegetation at elevated concentrations and it has a large influence on the chemical reactivity of the troposphere through its ability to generate hydroxyl radicals on photolysis. It is now becoming accepted that the quantity of ozone is increasing in much of the troposphere. According to reports at a recent meeting*, ozone levels are increasing over both the continents and the oceans in the Northern Hemisphere. Also, it seems, the various chemical precursors of ozone — hydrocarbons and nitrogen oxides — are now spread throughout the troposphere. Observations of substantial concentrations of hydrogen peroxide, whose precursors react with nitrogen oxides to give ozone, underline this view of tropospheric ozone.

Brühl and Crutzen's new work concerns the different effect that tropospheric and stratospheric ozone have on the

biologically active ultraviolet radiation (UVB) from the Sun. They argue that the path of light is less direct through the troposphere than it is through the stratosphere: this is because molecules, cloud droplets and aerosols, which are present in greater density in the lower atmosphere, scatter the light rays. The path length through the troposphere is thus relatively increased and each ozone molecule is more likely to intercept and absorb light.

The authors calculate the effect of this mechanism using data from the Hohenpeissenberg Observatory in Bavaria on the ozone concentration from 0–10 km (the troposphere) and above 10 km (the stratosphere). They find that between 1968 and 1982, the UVB reaching the Earth's surface actually decreased by 0.9 per cent at noon and by 0.5 per cent throughout the day. If a weighting factor is applied to the proportion of the UVB that interacts with DNA, the decreases calculated for noon and the whole day at the summer solstice become 1.7 and 0.9 per cent, respectively. This occurs in spite of an overall decrease in the ozone column density of 1.5 per cent, attributable to stratospheric depletion, over the same period. The contribution made by ozone in the troposphere is much more effective at lower solar zenith angles. At high solar zenith angles, which is the case for the winter months at high latitudes, the scattering of UVB mostly occurs in the traverse through the extended path length in the stratosphere.

Brühl and Crutzen emphasize the role played by increased absorption of ultraviolet by ozone throughout large parts of the remote troposphere. Its effect will be much more pronounced in the Northern Hemisphere where ozone concentrations have increased substantially over the past 50 years (S.A. Penkett in *The Changing Atmosphere* (eds F.S. Rowland & I.S.A.

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* *Tropospheric Ozone*, Norwich, 3–7 July 1989.

Isaksen) 91–103 (John Wiley, Chichester, 1987)). Their paper also counters suggestions that measurements indicating a decline in UVB in urban areas of the United States during 1974–1985 were unrepresentative because of the large scattering caused by aerosols in local pollution. In my opinion, sulphate aerosol concentrations (the principal scatterer) are unlikely to have increased in the urban

United States during the past 20 years. It is more likely that they will have remained constant or declined as they have in the United Kingdom (L. Salmon *et al. Sci. tot. Env.* **9**, 161–200; 1978) because of the increased use of cleaner fuels. □

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EVOLUTION

Generating novelty by symbiosis

John Maynard Smith

MOST biologists now accept that the evolution of eukaryotes from prokaryotes involved the symbiotic union of several previously independent ancestors: a host cell, a mitochondrial ancestor, a chloroplast ancestor and, more controversially but more importantly, a prokaryote that brought with it the structures that today provide cell motility — undulipodia — and that move things around inside cells — microtubules, centrioles and the spindle. We are also uncomfortably aware that the success of bacteria in defeating our attempts to kill them with antibiotics depends on their ability to acquire the necessary genes from elsewhere, usually on plasmids. What these two processes have in common is that genetic material from two very different ancestors is brought together in a single descendent. Did some, or even most, other major novelties in evolution arise in an analogous way? A number, at least, of those attending a recent conference* on symbiosis would answer in the affirmative. As one who was invited as a potential critic of this view, I will first summarize some of the facts that were reported, and then give my own opinion of their significance.

On a number of occasions, plants and animals have acquired important metabolic capacities through symbiotic union with prokaryotes, or with fungi. Termites digest cellulose with the help of a diverse population of protists and bacteria in their gut. Ericaceous plants are dominant on acid peat soils because of their relationship with a single species of mycorrhizal fungus, which makes available nutrients inaccessible to other plants. Aphids and leaf-hoppers can live on plant phloem because their cells contain bacteria able to perform syntheses that their hosts cannot (T. Tiivel, Estonian Acad. Sciences, Tallin); a similar relationship exists between some weevils and their intracellular bacteria (P. Nardon, Laboratoire de Biologie Appliquée, Villeurbanne), but in this case the insects can survive, albeit with reduced fertility, in the absence of the bacteria. The Leiognathid fishes use

light organs to assist vision, and to give signals (M. J. McFall-Ngai, U. Southern California, Los Angeles) and the light is generated by potentially free-living luminescent bacteria, although some other fishes generate light using enzymes programmed by their own chromosomal genes. Finally, the Pogonophoran worms that inhabit deep-sea vents (and also the Lucinid bivalves that live, less romantically, in the sewage outflow from Los Angeles) do not feed as adults, but rely instead on the energy supplied by endosymbiotic bacteria that oxidize hydrogen sulphide and use the energy to fix carbon (R. Vetter, Scripps Institute Oceanography).

This list, although incomplete, is impressive. Of course, not all symbiotic relationships are mutualistic. The most remarkable parasitic relationship described occurs in red algae (L. J. Goff, U. California, Santa Cruz). Often, a host species of red alga is infected by a parasite closely related to itself. The parasite

transfers a nucleus into the host cell, where it undergoes DNA synthesis and division, and in effect takes over from the host nucleus. It is tempting, but still speculative, to suggest that this situation has evolved from normal fertilization, with the male nucleus becoming parasitic on the female cytoplasm. However, it is the mutualistic cases that seem to offer the best hope of evolutionary novelty, and it is these that I shall discuss.

The first issue concerns 'gradualism', and the contrasting possibility of relatively sudden innovation by symbiosis. Here I see no serious contradiction. I am a gradualist because I do not believe in miracles. A single mutation can produce a large morphological effect, but it would be a miracle if it were to give rise to a complex adaptation. If I were told that a fish had acquired, in a single mutational step, the five genes that code for the enzymes that generate bioluminescence, I would not believe it. But I see no difficulty with the idea that a fish could acquire luminescence by symbiosis with a bacterium that already possessed the necessary genes. The problem of miracles, however, does not disappear. The bacterial genes themselves had to evolve, and they presumably did so by mutation and selection. Similarly, the complex light organs of the fish, with their reflectors and movable shutters, had to evolve, and again they presumably did so by mutation and selection: it would be a miracle if the mere presence of the bacteria induced, at a stroke, a complex structure of benefit to the fish. Nevertheless, the bringing together of genetic information from two unrelated sources can certainly generate novelty, as the

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REASONS

Flashlight fish (*Photoblepharon palpebratus*) of the Comores Islands, illuminating symbiosis.

*Symbiosis as a Source of Evolutionary Novelty, Bellagis, 25–30 June, 1989.

examples listed above illustrate.

A second question concerns the kinds of novelty likely to arise in this way. Prokaryotes and fungi are good at metabolic tricks that animals and plants have lost, or never had — an obvious example is vitamin synthesis. Symbiosis with them may provide new metabolic abilities, but is less likely to be relevant in the evolution of new modes of locomotion or feeding, which is what palaeontologists tend to have in mind when they talk of macro-evolution. I therefore doubt whether symbiosis has been important for the morphological evolution of plants and animals, except in so far as it may generate selection for morphological change, as in the example of luminescent organs in fish. Even if a symbiont induces a morphological change in its host (and examples of this were reported), the host response, if adaptively complex, has to evolve by natural selection.

A final problem concerns the selective mechanisms responsible for the origin of symbioses. Parasitic interactions present no special difficulty, but mutualism is more puzzling: why should a symbiont benefit its host if it could gain immediate advantage by injuring it? The decisive questions concern methods of transmission and multiplicity of infection. The bacterial endosymbionts of weevils and of plant-sucking bugs can live only in their hosts, and are transmitted in the host eggs. Hence a symbiont that damages its host reduces its own chances of leaving descendants. But most symbioses probably passed through a stage in which the

association had to be re-established in every generation. This is still the case for the other examples listed above. Larval Pogonophora have a mouth, and swallow their future symbionts. Young fish swallow luminescent bacteria, young termites must acquire their gut symbionts, usually through the rear end — this may be one reason why they become social — and seedling plants re-establish contact with mycorrhiza in every generation.

With this life history, the spread of selfish mutations, converting a mutualistic relation into a parasitic one, is always a possibility. Yet all the above examples are ancient. One possible explanation for their stability is that few selfish mutations are possible. For a fungus, a heather plant is an excellent supplier of fixed carbon, and it may be better to keep alive the goose that lays the golden eggs than to kill it. But this argument depends on the assumption that, if you do not kill the golden goose, no one else will either: that is, it assumes that the host is infected by a single clone of symbionts. The same argument applies to the evolution of parasites from greater to less virulence.

Further work is needed on the origin and maintenance of symbiosis, but it is already clear that, by bringing together genetic material from distantly related ancestors, symbiosis provides a source of evolutionary novelty that is additional to mutation and homologous genetic recombination. □

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ASTROPHYSICAL MAGNETISM

Stretch, twist and fold

H.K. Moffatt

DYNAMO action, in astrophysical contexts, is the spontaneous growth of magnetic fields caused by the motion of electrically conducting fluids. That fluid motion can sustain a magnetic field against its natural tendency to decay is well-established, but there are doubts about the rates at which magnetic fields grow when the conditions for dynamo action are satisfied. It appears still to be an open question whether there can be a smooth velocity field capable of generating a fast dynamo action, in the sense of the distinction¹ between 'fast' and 'slow' introduced by Vainshtein and Zeldovich² in 1972. That question, and the surprising connections that have emerged recently with the theory of iterated maps, were explored at a recent workshop*.

Dynamo theory is important in planetary physics and astrophysics because of the ubiquity of magnetic fields. Nearest to

home, the Earth's magnetic field is sustained by dynamo action in the outer liquid core, and the magnetic fields of Jupiter and Saturn and possibly of other planets are believed also to originate from fluid dynamos. If it were otherwise, these fields would decay in times of the order of 10^4 years, which is short compared with the age of the Solar System, throughout which, on geological and other evidence, the fields have persisted.

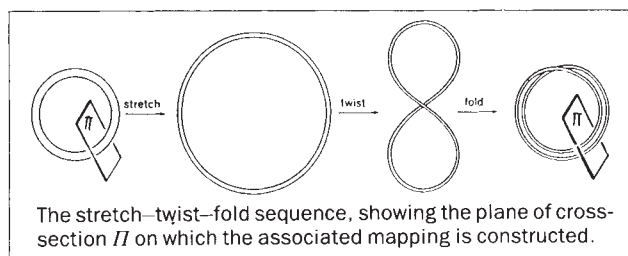
Stellar and, in particular, solar magnetism presents another problem. The natural decay time for a magnetic field may be 10^9 years, but there are systematic variations such as the 22-year solar sun-spot cycle on much shorter timescales. Rapid changes such as these, involving global reversals of magnetic polarity, must be attributable to fluid motion deep in the

turbulent stellar convection zone. Whether galactic magnetic fields also originate in dynamos is another matter³.

The property of fluid motion crucial to dynamo action is helicity — in simple terms, the net imbalance between right-handed and left-handed helical motion. Surprisingly, it has recently been shown⁴ that Ponomarenko's helical dynamo⁵, perhaps the simplest and purest of all, in which helical streamlines are confined to a cylinder, is a fast dynamo, but only when the velocity is discontinuous across the cylinder boundary. The other known model of fast dynamo action is that of Soward⁶, who has shown that certain spatially periodic velocity fields have the properties of a fast dynamo provided that there are mild singularities of vorticity at the stagnation points.

These examples are somewhat contrived, which may in part explain why the search for other more realistic examples has excited so much interest. The physical, rather than mathematical, prototype of a fast dynamo is that first discussed by Vainshtein and Zeldovich², and may be likened to the process of stretching, twisting and folding a rubber band so as to double the tension. A similar procedure applied to a magnetic flux-tube in a perfectly conducting fluid (see figure) will double the magnetic field-strength, so that indefinite repetition of the stretch-twist-fold sequence will lead to exponential growth of the magnetic field on a time-scale determined by that of the motion, and independently of molecular diffusion. This example, of course, is also somewhat contrived: but insofar as stretching is invariably associated with turbulent flow, twisting with convection and folding with geometrical constraints thereon, the stretch-twist-fold sequence has some basis of reality in the turbulent convection zone of a rotating star.

How can one translate this physical argument into mathematical form? The stretch-twist-fold sequence can be represented as a three-dimensional time-dependent velocity field, but the resulting equation for the evolution of the magnetic field is so complex that it cannot be solved even with available computing power. But now there is an alternative procedure. The stretch-twist-fold sequence doubles the magnetic field-strength without increasing the cross-section of the flux-tube, which is tantamount to a mapping of the cross-section onto itself.



*Workshop meeting at Observatoire de Nice, 25 - 30 June, 1989.

That observation alone links dynamo theory with the theory of iterated mappings, about which much is known. Several examples of iterated mappings, called dynamo maps, have now been found, notably by Bayly and Childress⁷ and by Finn and Ott⁸, and were scrutinized at the workshop. It emerges that, although every time-periodic flow yields a map and every continuous map can be associated with a flow, it is not yet known whether these flows have the property of 'orientability' that would lead to reinforcement (rather than cancellation) of the convected magnetic field. That fundamental problem remains an obstacle in attempts to tackle dynamo theory with the theory of iterated maps.

There remains the sledge-hammer of the high-speed computer. The velocity field most likely to be tractable, is called the 'ABC' flow — essentially a superposition of three orthogonal circularly polarized velocity fields such that the velocity field $\mathbf{u}$ has the components ($B\cos ky + C\sin kz$, $C\cos kz + A\sin kx$, $A\cos kx + B\sin ky$). This flow is named after Arnold⁹ (who recognised that the particle paths may be chaotic), Beltrami (who first conceived of flows such as this in which the vorticity is everywhere parallel to the velocity) and Childress (who recognized the flow, through its property of maximal helicity, as a prime candidate for dynamo action).

Galloway and Frisch⁹, following Arnold⁹ and Korkina¹⁰, have computed the dynamo growth-rate as a function of the magnetic Reynolds number R_m (a dimensionless measure of electrical conductivity), and find two windows of dynamo action, in the ranges $8 < R_m < 18$ and from $R_m = 27$ up to $R_m = 400$, the present limit of reasonably accurate computation. The challenging question still outstanding is whether the ABC flow generates a fast or slow dynamo, or no dynamo at all, in the limit in which R_m tends to infinity.

A novel twist to this question was raised at the meeting by B.J. Bayly (University of Arizona, Tucson), following work by A.A. Ruzmaikin (Moscow). If the three ingredients of the ABC flow associated with the parameters A , B and C are successively switched on and off at random, separated by periods when only diffusion operates, the result is a model reminiscent of the 'stasis' model of Backus¹¹, who provided one of the first proofs of dynamo action — in the event, a slow dynamo. Bayly has now shown that fast dynamo action is also possible and, interestingly, that the wavelength of the growing magnetic field is exactly twice that of the underlying ABC ingredients of the flow. This behaviour has escaped attention in previous computations, which have concentrated on magnetic fields with the same periodicity as the velocity field.

Bayly's result, if confirmed, is certain to stimulate further computational attacks on the ABC problem.

An explicit fast-dynamo theory is potentially of the greatest importance in astrophysics because present accounts of stellar magnetism are based on insubstantial foundations. Most modern theories follow the mean-field formalism introduced by Steenbeck, Krause and Rädler¹² in which the effects of turbulence in convection zones are parameterized by a generation coefficient α and a turbulent diffusivity β , which are usually related to the velocity and length scales of the turbulent convection by simple dimensional analysis. The resulting growth rates are determined by α , β and global properties such as differential and meridional circulation in the star, but do not depend on the magnetic diffusivity of the medium, so that the dynamos are intrinsically fast. Thus the whole theory of stellar magnetism hangs on the slender thread of dimensional analysis applied in a complex turbulent context. The mathematical difficulties inherent in fast dynamo theory are concealed in the mean-field theory which, in principle, determines α and β , but there is a paradox: the molecular magnetic diffusivity seems to be essential to the production of an α -effect even though the resulting value of α is independent of the value of the diffusivity, which is in any case vanishingly small.

If the solar dynamo is, indeed, a fast dynamo, then it is to be expected on dimensional grounds¹³ that the field will nearly everywhere have a fine scale of the order of R_m multiplied by L , the length-scale of the velocity field. Taking L to be of the order of 1,000 km from the observed granulation of the solar surface, and R_m to be of the order 10^4 , there should be variations of magnetic field on scales as small as 10 km, which is not incompatible with observation. Fast dynamo theory may therefore be of much more than merely academic interest. □

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PROTEIN STRUCTURE

One enzyme from two genes?

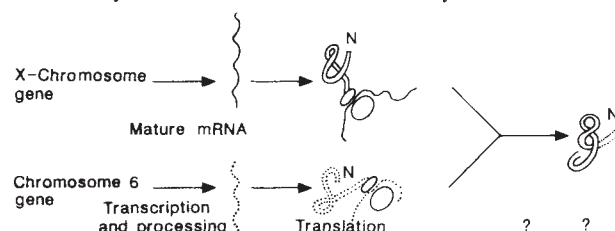
Lucio Luzzatto

GLUCOSE-6-PHOSPHATE dehydrogenase (G6PD) has long been a favoured system in human biochemical genetics. It was one of the first mammalian enzymes to be characterized¹, it is one of the most polymorphic genes in the human genome² and, because it maps to the X-chromosome (Xq28)³, it has served as a wonderful marker to study X-chromosome inactivation⁴, the consequent mosaicism of somatic cells in females⁵, and the clonality of tumours⁶. In addition, this mosaicism is probably responsible for the biological advantage of G6PD deficiency with respect to malaria⁷. Now, an entirely unexpected development, of potentially general significance, arises from a study of G6PD just published in *Cell*⁸. If the work is confirmed, we shall have to adjust to a new way of thinking about how proteins can be assembled.

Briefly, Yoshida and his colleagues find that whereas one form of G6PD in red cells has an amino-acid sequence that conforms to

that predicted by the cDNA sequence first published by Persico *et al.*⁹, another form differs upstream of amino acid 36. By the use of two synthetic oligonucleotides corresponding to portions of the divergent sequence, they have isolated human genomic and reticulocyte cDNA clones that encode this sequence as part of a protein of 345 amino acids, the gene for which maps to chromosome 6 rather than the X chromosome.

They must, of course, have considered the possibility that the G6PD preparation used for protein sequencing was contaminated. But the remarkable result, on which the rest of the paper depends, is that the authors have actually isolated from a



Can a hybrid protein be formed during or after the translation of separate gene transcripts? And, if so, how?

red-cell G6PD preparation a tryptic peptide of 21 amino acids which, on sequence analysis, consists of 13 amino acids encoded by the chromosome 6 gene followed by 8 amino acids encoded by the X-chromosome gene. From this, Yoshida and colleagues infer that the major human-red-cell G6PD consists of an N-terminal portion of 52 amino acids encoded by a chromosome-6 gene, attached to 479 amino acids encoded by the X-chromosome gene.

Unprecedented

How can such an unprecedented hybrid protein be formed? Attempts to isolate a hybrid cDNA (which might have resulted from *trans*-splicing of two separate primary transcripts) must have been strenuous, but were not successful: indeed, probes belonging to the two genes light up mRNA species of different sizes. Therefore, the authors are led to the conclusion that splicing of the two molecules must take place either at the stage of translation (ribosome 'hopping'), or at a post-translational stage (transpeptidation). Barring the unfortunate eventuality of transpeptidation having taken place as an artefact *in vitro*, no other explanation seems possible.

Of course, this raises a host of questions. For instance, what cells have the transpeptidation machinery, and how is its specificity guaranteed? What is the fate of the C-terminal portion of the chromosome 6 protein, and of the N-terminal portion of the X-chromosome protein after transpeptidation? Does the full chromosome 6 protein exist, and what is its function?

Whatever the answers to these questions may be, the biological significance of site-specific transpeptidation, if it occurred generally, would be of considerable magnitude. As far as I know, the only precedents are the addition of ubiquitin to proteins before their degradation (but in this case an *isopeptide* bond is formed through an ϵ -amino group on lysine), and the post-translational processing of concanavalin A (but in this case portions of the same translation product, rather than two different translation products, are ligated together¹⁰). Despite introns, and despite the many variations on the theme of alternative splicing, recombination at the RNA level has not made any serious dent in the concept of the mendelian gene: recombination at the polypeptide level certainly would.

Synthesis

Coming back to G6PD, there are four reasons that argue against the chromosome 6 gene being of major importance with respect to synthesis of the enzyme. First, human G6PD made in rodent-human somatic cell hybrids containing the X as the only human chromosome is

indistinguishable from normal human G6PD¹¹. Second, G6PD produced in COS cells¹² from a construct containing that cDNA is indistinguishable by electrophoresis from human-red-cell G6PD. Third, G6PD produced in *Escherichia coli*¹³ using a similar construct is (from our unpublished results) also indistinguishable in size, charge and kinetics from human-red-cell G6PD; therefore, if the G6PD produced by transpeptidation is the principal species in red cells, one would have to conclude that, despite its different size, net charge and N-terminus, its physical and enzymic properties are somehow identical to the G6PD encoded by the X-chromosome gene alone. Finally, there is an evolutionary argument: because Yoshida and colleagues find that the chromosome 6 gene is apparently limited to primates, whereas G6PD as a whole is highly conserved in evolution, what is the unique role of the N-terminal portion of the protein in the red cells of primates?

In the past two years, analysis of G6PD variants identified from clinical and population studies has begun to give us a picture of the molecular basis for the 300 or so alleles of this locus. Thus far, only point mutations have been observed¹², and none has been in exons I or II. The accumulation of more data on mutants may help us to understand why the gene is so polymorphic (is it also more mutable?), and to pinpoint functionally important domains. The fact that no deletions have turned up supports the biased view of G6PD fans that this is not just a house-keeping gene but is actually indispensable for house management. The startling report by Yoshida's group could set us up for the ultimate surprise: a G6PD deficiency that will be autosomal instead of X-linked. □

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High impurity

ATMOSPHERIC chemistry is very perverse. At ground level, ozone and nitrogen oxides are pollutants. Ozone forms photochemical smog and nitrogen oxides contribute to acid rain. Twenty kilometres up, however, they are transformed into goodies. The high ozone layer is formed by, and protects us from, the far-ultraviolet component of sunlight. And nitrogen oxides help to protect the ozone layer. For stratospheric ozone is catalytically decomposed by halogen radicals, perversely formed from the halocarbon gases which are so benign and useful at ground level. Just one such halogen radical can destroy thousands of molecules of ozone in an efficient chain reaction. Nitrogen oxides, formed from the stratosphere's small content of nitric acid, combine with and deactivate these radicals. So Daedalus, in the cause of environmental purity, is planning to inject large amounts of nitrogen oxides into the stratosphere.

The cure may seem worse than the disease — acid rain is more damaging than ultraviolet light. But Daedalus will inject his oxides indirectly, as ammonia gas. This reacts with ozone to give nitrogen oxides. Each ozone molecule lost in the oxidation will save thousands of its fellows from destruction.

The great advantage of this indirect approach is that ammonia is lighter than air. In principle you could loft it to the stratosphere in big balloons. Daedalus's plan is even more elegant — he is scaling up vortex-ring technology. A vortex ring, of course, travels as a self-contained parcel of gas, and exchanges very little with its environment. Furthermore, a vortex ring of buoyant ammonia would have inherent lift. It would not slow and dissipate like a smoke-ring, but would rise to a great height like a sort of skinless balloon. DREADCO chemical engineers are building huge pulse-pipes from which big vortex rings of ammonia can be fired upwards at the stratosphere. They are seeking the ideal size and velocity for an ammonia ring that shall just reach the ozone layer before turbulence and diffusion break it apart. Some of its ammonia is bound to escape into the atmosphere on the way up, to be washed out again by rain. Ammonia-rain, of course, would be alkaline — a useful counter to acid rain.

This elegant scheme has many advantages. Ammonia is one of the few chemicals made in tonnages vast enough to have a significant effect on the atmosphere, even in the modest catalytic role here proposed. Sooner or later it will come down again, adding to the precipitation of combined nitrogen and helping to fertilize the planet at large. As ammonia is mainly used as a fertilizer anyway, the ground-level economy will remain largely unaffected.

David Jones

Safety in the laboratory

SIR—We wish to report briefly on two matters of laboratory safety that have been raised recently in Scientific Correspondence.

The first concerns the claim by Arnold *et al.*¹ that the existence of channels and pores in latex gloves revealed by scanning electron microscopy raises doubts about whether such gloves are effective barriers to viruses. It was subsequently pointed out that these channels may be artefacts of sample preparation² and that there is no evidence that viruses are able to pass through latex gloves^{3,4}. We have now found that gloves worn in clinical and research laboratories can be readily penetrated by a bacterial virus — bacteriophage lambda.

We compared the ability of gloves made of thin polyethylene (PE), polyvinyl chloride (PVC) and latex to be penetrated by this virus both before and after exposure to the disinfectant ethanol (70 %). Sections of the gloves that cover the palm were tested under static conditions. Gloves of PE and PVC failed to be effective barriers to the virus in 40 % and 22 % of trials, respectively, rising to 94 % and 56 % after exposure to alcohol. The trials using latex gloves had a failure rate of 1–2%. Although this study did not involve specific human pathogens, it serves as a warning to those selecting gloves for handling biohazardous materials and illustrates the need for better glove testing standards and quality control.

Our second report is in response to the findings^{5,6} that many solutions of ³⁵S-methionine release volatile radioactive component(s), which can present containment problems. We have not found incubators used for ³⁵S-labelled compounds to be as highly contaminated as those reported⁵. We studied the volatility of ³⁵S-methionine under controlled conditions in open and closed containers. After incubation for 4 hours in closed containers, only 0.01–0.1 % of the ³⁵S was in volatile form. Although the volatile component was readily soluble in water, its loss — a function of the volume incubated and the vapour pressure — was independent of the evaporation of water from the incubation medium. Reliance on charcoal filters to trap the volatile ³⁵S-compound⁵ is unwarranted as binding of the compound to such filters was found to be non-quantitative and reversible.

The release of volatile ³⁵S-component(s) can be controlled by incubating culture vessels in a sufficient volume of media (14 ml mCi⁻¹) in an incubator with high humidity to reduce the evaporation rate of the incubation media, or by using a CO₂-gassed and sealed flask, or by using Petri dishes in sealed plastic bags. Tricine (50 mM), an effective stabilizer, should be

added to the media if it is not toxic to the biological agents in culture.

More detailed reports of our findings have been submitted for publication elsewhere.

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Periodical cicadas

SIR—Martin and Simon¹ suggest that 13-year cicadas in a large region of central North America are descendants of 17-year cicadas that switched their life-cycle length to 13 years. Their proposal that brood XIX in Illinois, Iowa and Missouri represents a permanent four-year acceleration from brood X that took place in 1868 is not supported by the historical record.

As Martin and Simon¹ point out, four-year accelerations are common in periodical cicada life cycles. But one-year decelerations are also known². I have analysed over 1,500 historical records of periodical cicada emergences dating back to 1804 in Illinois, Indiana, Kentucky and Ohio for patterns of emergence in a given locality. The results show that over 98 per cent of the records are for known broods, four-year accelerations or one-year decelerations from established broods. Moreover, in May/June 1988, two counties in southwestern Ohio and two counties in northern Kentucky witnessed an unexpected emergence of periodical cicadas. The emergence was as heavy as brood X which emerged in the region a year earlier and involved all three species. Examination of the hatch rate indicated it was as high as brood X, a long-established brood, possibly fore-telling of a future brood which would be numbered brood XI. The brood was heaviest in an area where brood XIV is the predominant brood. Moreover, brood XIV also experienced a one-year deceleration in 1975 in the same area which produced the 1988 emergence. If the unexpected 1988 periodical cicadas were derived from brood XIV it would suggest that the 1988 emergence was a four-year acceleration from the 1975 one-year deceleration. This combination of a four-year acceleration with a one-year deceleration would explain areas, such as northeastern Ohio, where adjacent broods V and VIII occur

with three-year differences in their emergences.

Historical records will not indicate mechanism, but they can give important evidence for different models. Lloyd and coworkers³ have proposed a mendelian model for four-year accelerations involving a hybridization between 17- and 13-year cicadas. But the Ohio history shows four-year accelerations in regions where only 17-year cicadas occur. Twelve counties in Illinois and much of southwestern Missouri recorded periodical cicada emergences in 1829, 1842 and 1855 indicating that the 13-year life cycle of brood XIX in Illinois and Missouri was established before 1868 (ref. 4). This does not speak against the proposed mechanism presented by Martin and Simon, rather it suggests that the acceleration that gave rise to brood XIX may have occurred earlier than they thought.

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SIMON AND MARTIN REPLY—The complex patterns of periodical cicada emergences are indeed mind-boggling as Kritsky points out. His painstaking historical analyses have shown that despite their much touted reputation, periodical cicadas are not as regular as clockwork. Nevertheless, the irregular emergences are far from random, being either one or four years out of phase. Furthermore, Kritsky's historical data base has proved invaluable in interpreting genetic patterns^{1,3,5}. For example, as he notes, hybridization between 13- and 17-year cicadas is not, as proposed³, a necessary condition for an acceleration in development.

We¹ have presented data which suggest that, in the midwest, a region that had been occupied by both 13- and 17-year cicadas is presently inhabited exclusively by cicadas possessing a 13-year life cycle but a 17-year genome. These cicadas are genetically identical to 17-year brood X which disappeared from the area in the first half of this century. Our conclusion is that midwestern 13-year cicadas of brood XIX originated from 17-year brood X by a four-year acceleration in development and joined an existing 13-year brood XIX which abutted it to the south.

We agree with Kritsky that the proposed four-year acceleration may not have occurred in 1868 but the presence of 13-year cicadas in the area before 1868 does not rule out this hypothesis. Indeed, a four-year acceleration of midwestern 17-year cicadas to produce 13-year cicadas could have occurred more than once. Despite the particular pathway that produced the geographical and temporal pattern we observed, both Kritsky's historical data and our genetic data indi-

cate that year-class formation occurs as a consequence of changes in life-cycle length.

The question that now needs to be addressed is, are these pre-1868 cicadas genetically 13-year or 17-year? If we can obtain alcoholic or dried specimens from 1829, 1842 or 1855, we can determine their mitochondrial genotype through DNA amplification and sequencing as we have done for other preserved periodical cicadas (Simon and Pääbo, unpublished data). A search is underway; anyone with

information on such specimens is urged to contact us.

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Parasites and sexual selection

SIR—Recent discussions about the prevalence of blood parasites and colour in birds^{1,2} have resurrected a debate which began in 1982 (ref. 3) but aroused little interest among parasitologists who had hoped that the whole matter would die quickly and quietly. In my view, all this work is strong on evolutionary theory and statistics but so weak on basic parasitology that the fundamental basis upon which the edifice has been erected is suspect.

The basis of the work has been published data on parasite numbers in stained blood smears, which are notoriously difficult to interpret even by experts. Overall, a single blood smear presents only a tiny window through which real infection can be glimpsed. It requires an almost super-human effort to examine the whole of a blood smear properly, so short cuts are usually taken⁴. Furthermore, given the many factors that affect infection, the true prevalence of any blood parasite cannot be determined by single blood smears. It requires several sequential smears taken over a long period of time to be fairly sure, and an immunological or molecular test to be virtually certain that a host is infected with a particular parasite. In this context, Zuk² finds it intriguing that rarely trapped birds show a stronger correlation between brightness and parasites more than commonly caught birds. The explanation for this is simple — and here I plead guilty — for when examining blood smears, one takes much more care if they come from rarer birds because these are more likely to produce observations justifying publication.

We know practically nothing about the pathology of many blood parasites that infect birds, and what we do know comes largely from laboratory observations or anecdotal evidence. Some parasites can kill their hosts, but some hosts exhibit a phenomenon known as premunition during which a tolerable infection prevents the establishment of a less tolerable one. Furthermore, other concomitant infections cannot be ignored because blood parasites are markedly affected by the

presence of other parasites, bacteria or viruses. Practically nothing is known about immunity to parasites in passerines and it is futile to try to make any assumptions about the ways in which these blood parasites affect the lives of their hosts.

It therefore does not seem surprising that there is no agreement as to whether there is any connection between the colour of birds and the presence of blood parasites. To a parasitologist, the only suggestion that makes any sense is that of Read and Harvey¹ who suggest a phylogenetic relationship. It would be interesting to see this followed up in a systematic way.

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HAMILTON AND ZUK REPLY—Before responding to Cox, it is necessary to deal with inaccuracies in Read and Harvey's response¹ to Zuk². Her claim (on which both of us, as authors of the original paper³, agreed) was that Read and Harvey's re-analysis⁴ in fact produces significant correlations between male brightness and parasite prevalence in passerine birds. This is not wrong. Read and Harvey¹ evidently mean that it would be wrong to claim such correlation after their procedures for removing 'taxonomic association' have been applied, but Zuk's letter was not referring to this case but to ordinary cross-species comparison, as in our original paper³. Almost every dataset has some potential classification underlying it which has not yet been 'controlled'. It is not wrong to say that a study on a school class reveals a 'significant correlation of exam mark with writing speed' just because there are several ethnic groups in the class. 'Controlling' an underlying complex of relationships in such a case may clarify and it may also deny evidence of an important cause. However, even had we made the claim that Read and Harvey criticize we would still be half right: their

report tells that on the side of the Atlantic where the new scorers were treating familiar birds the association within taxa had $P=0.008$.

They seem to reject this because it reduces if species with small sample sizes are excluded. That new fact is very interesting but does not contradict either our actual claim or the stronger one they treat as made. As to reasons, we suggested one which Harvey and Read reject. Whether that argument is wanting or not, a point not clear to us, another that can be independent or auxiliary is confidently suggested by Cox in his letter in this issue. Thus, it now seems that the most carefully assessed bird species — those rarely caught, on Cox's authority — after scoring by local ornithologists and statistical removal of taxonomic artefact, accord very well with our expectation^{4,5}. Thus the combination of data and analysis which all five contributors to this correspondence most nearly agree to be valid rejects no association with $P=0.003$ (two-tailed) and shows association in the direction we predicted⁴.

That more infections are apparently added for showy birds than for dull when smears are carefully searched is also interesting. Low-level infections in showy birds fit well with theory because sexually selected species, co-evolving faster, may be better able to keep them low. Read and Harvey also suggest this in a different context, claiming we discounted the possibility. We did not, although we do expect sexual selection only to be able to diminish parasite incidence, not eliminate species.

Cox's comments on the weaknesses of the data are similar to those we found in most of the original papers. However, all authors who reported data had enough faith in their methods to think it worthwhile to tabulate numbers of birds caught and cases found. Provided there is no reason to expect a capricious relationship between observed and true incidence our test can proceed. It is remarkable and reassuring that from such weak data all the 'showiness' calculations, now done for three continents with contributions from three sets of scorers, have uncovered correlations of the same type.

We share Cox's wish for better understanding of the parasite conditions. We would especially like to see chronic virus conditions included in cross-species surveys. We do not agree with Cox, however, that it is futile to assume that passerine parasitaemias are usually detrimental, even though some studies on particular genera have failed to show this⁶. Both the known immense impact of disease associated with parasitaemia in humans and domestic animals, and a well-supported theory of how virulence might be maintained when parasites have mobile vectors⁷, reinforce this assumption. Even if it is true that certain infections can be

tolerated by many individuals, this does not remove these from the ambit of our theory. Indeed, a tentative connection between commitment to symbiotic microbes and sexual selection is indicated in a variety of disparate taxa from anelgesid mites to ruminant ungulates⁸.

We also join Cox in hoping that the 'phylogenetic relationship' found by Read and Harvey⁹ ('association' in their terms) will be followed up. Its slant is consistent in the studies carried out and suggests to us the following possibilities in accord with our thesis: first, parasites bring high sexual selection, and this, through stringent mate choice, encourages speciation⁹⁻¹¹; second, non-conforming trends (costly sexual selection with no payoff to descendants' health, or no sexual selection when parasites are damaging) reduce species survival.

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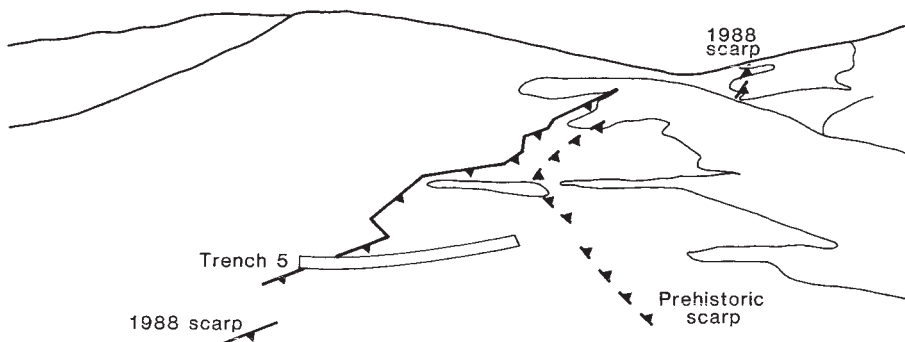
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Armenian earthquakes

SIR—A recent report¹ on the 1988 Armenian earthquake raises important issues concerning earthquake hazard and tectonic deformation rate in this region. The figure shows part of its fault scarp, indicating what seems to be an older Holocene scarp sub-parallel to it. Assuming that the local soil formed near the end of the Pleistocene glaciation (~10,000 years ago,) the estimated recurrence interval of major earthquakes on this fault is about 5,000 years or more, and the 2-m Holocene throw implies an average slip rate of ~0.2 mm yr⁻¹ or less. The 1988 earthquake occurred on one of a set of parallel reverse faults with ESE strike, some of which had been identified previously^{2,3}, that take up shortening across Armenia and the adjacent Soviet Transcaucasian republics. The largest known historical earthquake in what is now Soviet Transcaucasia occurred in 1668 near Shemakha⁴⁻⁶, now in



The view looking WNW from latitude 40°49.9' N longitude 44°13.1' E showing, highlighted by shallow snowdrifts, the 1988 fault scarp at ~1,900-m elevation, ~500 m east of its highest point, and the suggested older scarp up to 10-m farther north to its right, both with ~1-m reverse throw. Both scarps can be followed along strike for ~500 m in soil that is probably late glacial or early post-glacial (~10,000-yr old), given the presence of moraine deposits and glacial cirques at similar elevation nearby. The arid climate and the absence of local soil disturbance by agriculture (in contrast with lower elevations) favour scarp preservation. The abrupt 1988 scarp has been trenched, but the trench stops ~2-m short of the other, more rounded, scarp. It is hoped that this trench will be extended northward and radiocarbon dating of samples from it will provide quantitative constraint on the Holocene slip history suggested for this fault. Both scarps probably merge into the same active reverse fault at a few tens of metres depth, which dips NNE (to the right) at ~60°. The photograph was taken in February 1989. The sketch shows an interpretation, with chevron markers indicating the hanging walls of both scarps which are uplifted relative to the footwalls.

Soviet Azerbaijan. The ~50-km WNW-ESE extent of severe damage, more than in 1988 indicates M_s (surface-wave magnitude) ≈ 7 . Including the 1988 event ($M_s = 6.7$) only five Transcaucasian earthquakes in the past 1,200 years, or so, are known⁶ to have caused damage equivalent to $M_s 6.5-7$ (ref. 6). Co-seismic slip rate on other individual faults must, therefore also be very low, and the shortening rate across Transcaucasia can be no more than a few millimetres a year⁷. Nonetheless, it seems prudent to base regional earthquake hazard planning on the assumption that an earthquake with $M_s \geq 7$ may occur on any of these faults, with a probable recurrence interval for such events on any individual fault of many thousands of years. Furthermore, the suggested low

regional deformation rate means that many geomorphological features such as river terraces identified¹ in Armenia and believed to be related to active faulting are probably far too old to be radiocarbon dated.

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Global patterns of tectonic stress

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Regional patterns of present-day tectonic stress can be used to evaluate the forces acting on the lithosphere and to investigate intraplate seismicity. Most intraplate regions are characterized by a compressional stress regime; extension is limited almost entirely to thermally uplifted regions. In several plates the maximum horizontal stress is subparallel to the direction of absolute plate motion, suggesting that the forces driving the plates also dominate the stress distribution in the plate interior.

Two primary categories of forces are responsible for the state of stress in the upper, elastic part (reaching to depths of 10–35 km) of the Earth's lithosphere. The first category is responsible for what we shall call here 'tectonic stresses'. These broad-scale forces include plate-boundary forces (which either drive or resist plate motion), forces resulting from geodynamic processes (including broad-scale flexure of the lithosphere owing to surface and subsurface loads and/or inhomogeneous density distributions), and thermoelastic forces in cooling oceanic lithosphere. The second category is derived from local effects of topography, anisotropy of strength or elastic properties, and effects of erosion and man-made excavation; these forces generate local or 'induced' stresses. Spatial uniformity of the *in situ* stress field generally allows us to distinguish easily between these two categories. For example, tectonic stresses typically are uniform over distances many times (from 2–3 times to more than 100 times) the thickness of the elastic part of the lithosphere, whereas local stresses have wavelengths that are a fraction of this thickness. Induced stresses are critical in many mining and civil-engineering situations, but tectonic stresses provide valuable constraints on a variety of broad-scale geological problems such as the relative magnitudes of plate-driving forces and the nature of deformation and seismicity in the plate interior, far from plate boundaries (so-called intraplate or midplate deformation).

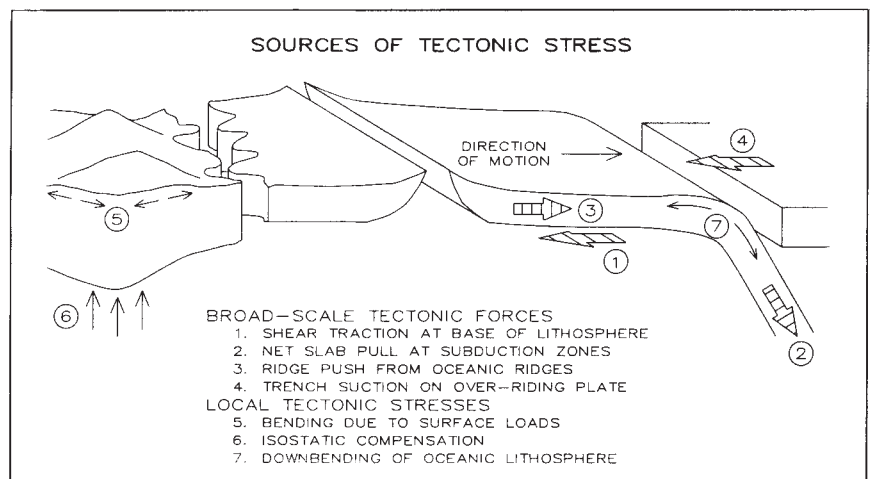
The World Stress Map project of the International Lithosphere Program is compiling a global database of contemporary *in situ* stresses in the crust using a variety of geophysical and geological measurement techniques. The project is a collaborative effort which at present involves over 30 scientists from 19 different countries. This project builds on early investigations of stress patterns in central and eastern North America and in western Europe, which indicated that both horizontal stress orientations and relative stress magnitudes are uniform over broad regions of the Earth's crust^{1–10}. A comprehensive compilation and analysis of the state of stress of the contiguous United States¹¹ which used both geological and geophysical stress indicators, confirmed the regionally uniform stress orientations found in the earlier studies and also concluded that stress orientations inferred from different types of indicator were generally consistent, despite the variety of techniques being used and the different ranges of depth being sampled.

Here we focus on these regionally uniform, broad-scale stress fields in the lithosphere. The most probable sources of such stress fields are

the major forces acting on lithospheric plate boundaries. These forces include: shear tractions at the base of the lithosphere that can either drive or resist plate motion; a 'slab pull' force which represents a balance between the negative buoyancy of the subducted oceanic lithosphere, viscous drag on the subducting slab and frictional resistance to subduction at shallow depth; resistance to continent–continent collision; a 'ridge push' force resulting from the excess thermal elevation of young oceanic lithosphere; and a 'trench suction' force exerted on the overriding plate at subduction zones, which is caused by trenchward extension of this plate to fill the gap that results from the falling back of the subducted slab (see refs 12–19 for detailed discussions of these forces).

Geological evidence, thermodynamic considerations¹⁷ and relatively short timescales for changes in plate motion all suggest that viscous drag at the base of the plates resists, rather than drives, plate motion and probably balances the torque caused by driving forces on individual plates^{19,20}. Drag forces thus tend to act antiparallel to the direction of 'absolute' plate motion relative to the lower mantle. Slab-pull, trench-suction and collisional-resistance forces all act normal to plate boundaries. The ridge-push force is an integrated force acting over the cooling oceanic lithosphere and generally acts normal to the plate boundary, except where spreading is oblique to the ridge. Here we shall approximate the orientation of the ridge-push force within a plate by the local orientation of relative motion calculated from the pole of opening for mid-ocean-ridge segments.

More localized sources of tectonic stress include flexural (bending) stresses caused by surface loads supported by the strength of the lithosphere (for example, resulting from sediment



deposition or volcanic loading, mountain building and erosion), by subsurface loads required for isostatic compensation of topography (that is, adjustment of lithospheric density in response to variability of overlying load), by down-bending of the oceanic lithosphere in subduction zones, and by membrane stresses generated by the motion of plates over an Earth of varying radius of curvature²¹. From a global perspective perhaps the most significant of these forces is the combined effect of the surface load and the buoyancy of a low-density region (thickened crust and/or thin mantle lithosphere) supporting a region of high topography. If the buoyancy forces dominate they can produce a broad zone of deviatoric tension beneath major mountain ranges and high plateaus^{18,22,23}.

As indicated previously, lateral variations in strength or elastic properties can result in local induced stresses. However, in some cases these variations are so profound and are of such long wavelength that they dominate the tectonic stress field. The best documented example of this effect is an apparent $\sim 50\text{--}60^\circ$ rotation of the horizontal stresses in a 100–125-km-wide zone on either side of the right-lateral San Andreas fault in California, the boundary between the Pacific and North American plates. It is suggested that this rotation results from an extremely low shear strength of the San Andreas fault and the slightly convergent relative motion between the two plates^{24,25}. Geological and focal-mechanism data²⁶ suggest that a similar stress rotation occurs adjacent to the right-lateral strike-slip Alpine fault in New Zealand, the plate boundary between the Pacific and Indian-Australian plates.

Stress measurement

A variety of data indicate that the three principal stresses in the Earth's upper crust lie in approximately horizontal and vertical planes¹¹, as suggested by E. M. Anderson²⁷. Thus, the orientation of the principal axes of the stress tensor can be constrained by specifying the azimuth of one of the horizontal principal stresses. Absolute stress magnitudes at depths of earthquake generation are extremely difficult to obtain and it is generally possible to constrain relative stress magnitudes only from the style of active faulting or deformation occurring in a region (see box).

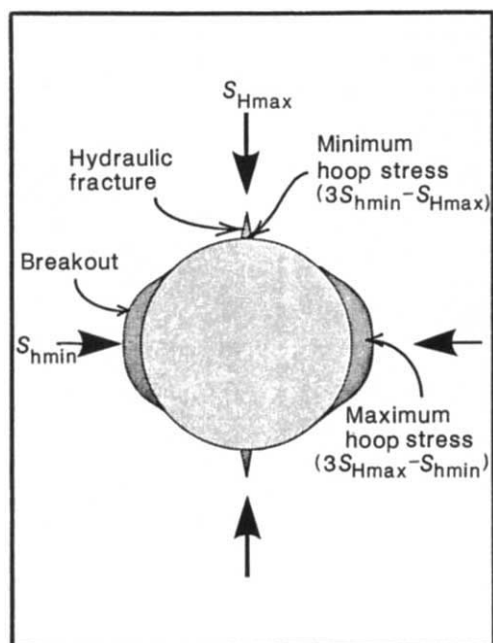


FIG. 1 Stress concentration around a vertical circular hole in an elastic half-space subjected to unequal far-field horizontal stresses, S_{Hmax} and S_{Hmin} . As indicated, breakouts and hydraulic fractures form as a result of these stress concentrations.

■ *In situ* stresses in the elastic part of the Earth's lithosphere are compressive (below the upper tens of metres).

■ Principal stresses lie in approximately horizontal and vertical planes:

$$S_V = \text{vertical stress} \approx \rho g z$$

$$S_{Hmax} = \text{maximum horizontal stress}$$

$$S_{Hmin} = \text{minimum horizontal stress}$$

■ Relative stress magnitudes determine type of deformation:

$$\text{Normal faulting: } S_V > S_{Hmax} > S_{Hmin}$$

$$\text{Strike-slip faulting: } S_{Hmax} > S_V > S_{Hmin}$$

$$\text{Thrust or reverse faulting: } S_{Hmax} > S_{Hmin} > S_V$$

Four different categories of geophysical and geological data are used at present as reliable indicators of horizontal stress orientations: earthquake focal mechanisms, stress-induced well-bore enlargements or 'breakouts', *in situ* stress measurements, including hydraulic fracturing and stress-relief measurements, and young geological deformational features, including both fault-slip and volcanic alignments.

A quality ranking system was developed recently²⁸ to aid in the analysis of the tectonic stress field in the lithosphere. This system permits a comparison of data collected by these different techniques and, in particular, aids in evaluating the significance of data in areas of sparse coverage. The ranking criteria are based both on the accuracy of individual determinations and on the relative reliability of the technique as an indicator of tectonic (as opposed to local or induced) stress. Four qualities are used, ranked in the order $A > B > C > D$. An A quality is assigned to data that record the tectonic stress field to within $\pm 10\text{--}15^\circ$, a B quality to within $\pm 15\text{--}20^\circ$, and a C quality to within $\pm 25^\circ$. In some cases D-quality data may record the *in situ* stress field reasonably accurately but because of a variety of potential perturbing factors (such as topography and free-surface effects) these data are not generally considered to be reliable indicators of tectonic stress.

These four main types of stress indicator have been described previously^{2,11,19,28}. The major recent advances that have made possible the compilation of global stress data are as follows.

Borehole breakouts as a reliable indicator of horizontal tectonic stress orientation. According to elastic theory, the maximum compressive circumferential stress developed around a vertical borehole is centred on the azimuth of the least horizontal far-field stress (S_{Hmin}), and the minimum stress occurs in the direction of the maximum horizontal stress (S_{Hmax})²⁹ (Fig. 1). Well-bore breakouts are a natural phenomenon that result from shear fracturing in the region of maximum stress amplification^{30,31}; they cause a cross-sectional enlargement of the hole in the direction of S_{Hmin} . In contrast, hydraulic fracturing is an active technique in which a portion of the well-bore is isolated and pressurized by injected fluids until a tensile fracture develops in the direction of S_{Hmax} . The orthogonality of breakouts and hydraulic fractures within a single well has now been confirmed by down-hole experiments in several wells in various tectonic settings^{31–33}.

Numerous investigations have established that consistently oriented breakouts form within an individual well and in wells within a given field^{32–39}. This possibility of multiple determinations in an individual well, and the ability to check for regional consistency among numerous wells, make breakout data valuable indicators of stress orientation. In addition, because wells for petroleum exploration and production are often drilled to depths of 3–4 km (and in some areas, up to 5–6 km), breakout data help to bridge the gap between the near-surface stress indicators (which sample typically less than 1 km depth) and

earthquake focal mechanisms (of typically 5–15 km depths). As can be seen in Fig. 2, there is generally an excellent agreement locally between stress orientations determined from well-bore breakouts and those inferred from earthquake focal mechanisms^{25,28}.

Resolution of earthquake focal mechanisms. Largely as a result of waveform modelling made possible by digital seismic acquisition networks, more and better-constrained focal mechanisms are now available. In addition, routine moment-tensor inversion of global network events⁴⁰ has greatly increased the database. An inherent difficulty remains, however, in estimating principal stress directions from focal mechanisms^{41–43}. Focal mechanisms are constructed from the radiation pattern of seismic waves, which defines a set of two possible orthogonal fault planes and slip vectors. P and T axes correspond to the maximum shortening and extensional strain directions for these shear faults.

But most crustal earthquakes are presumed to occur on pre-existing faults, in which case the fault-slip vector is a function of the orientation of the pre-existing fault and of both the orientations of the principal stresses and their relative magnitudes^{41,44–46}. Because of the uncertainty in inferring stress directions from earthquake focal mechanisms, a single mechanism, regardless of how well constrained, does not receive an A-quality ranking. This highest ranking is reserved for stress directions determined from mean P- or T-axis orientations or formal inversions for best-fitting stress axes^{47–51} of groups of moderate-sized earthquakes (at least one event with magnitude >4.5) occurring within close geographical proximity and with a variety of focal mechanisms. When using average P and T axes it is commonly assumed that errors resulting from slip on pre-existing planes of a variety of trends tend to cancel statistically^{11,52}. In general, aftershock sequences are not used because in some cases they appear to record deformation that is a secondary adjustment in response to slip in the main shock^{53,54}. A number of cases, however, show no discernible differences in stress orientation and relative magnitudes before and after the main shock^{55,56}.

Inversion algorithms to infer deviatoric stress tensors from fault slip and focal mechanism data^{46–51,57–59}. The underlying assumption of these inversion algorithms is that the measured slip vectors represent the direction of maximum resolved shear stress on the fault planes^{44,45}. Slip vectors, determined from striations observed on small fault planes of a variety of attitudes along active fault zones, are then inverted to yield a best-fitting deviatoric stress tensor. The inversion methods for fault-slip data^{48,57–59} are essentially the same as those used for focal-mechanism data^{47,49–51} and, in both cases, yield principal stress orientations that are generally well constrained⁵⁰. However, fault-slip inversions yield more precise information on relative stress magnitudes, because the true fault plane is known. The validity of this technique has been established by numerous studies which have demonstrated a reasonable match between predicted and observed slip vectors⁴⁸.

Often, particularly for historical earthquakes, only the overall slip vector and general attitude of the fault plane are known. In this case the data are treated as a palaeo-focal mechanism and the stress directions are inferred by the method of Raleigh⁴². These data are generally given a B-quality ranking. C-quality fault-slip stress determinations are estimated from the general attitude and primary sense of offset (but not actual slip vector) on an active fault plane.

Volcanic alignments as reliable horizontal stress indicators⁶⁰. Volcanic feeder vents such as dykes and cinder cones propagate perpendicular to the minimum principal far-field stress as natural, large-scale hydraulic fracturing experiments⁶¹. Thus, the mean strike of vertical dykes corresponds to the direction of S_{Hmax} . To study the current state of stress one uses vents of Quaternary age; however, palaeo-stress can be similarly studied using older dykes or cinder cones that are radiometrically dated.

Critical evaluation of stress relief measurements. One of the earliest and best studied techniques for investigating the state of stress in the Earth's crust is stress-relief measurements, commonly referred to as 'overcoring' measurements. This technique involves determination of the natural three-dimensional deformation (strain) relieved in a body rock *in situ* when separated from the surrounding rock volume^{62,63} and is the only technique that measures the complete stress tensor. Amongst the primary drawbacks of this method are that the measurements must be made near a free surface (either the Earth's surface or an excavated surface in a mine), that the strain relief is determined over areas of only a few mm² to cm², and that the change of stress on overcoring must be calculated from the strain relief, which requires knowledge of the complete compliance tensor. In particular, near-surface surface-stress-relief measurements have been shown to be subject to effects of local topography, rock anisotropy and natural fractures⁶⁴. Because of these difficulties, we have assigned a D quality to all near-surface (<5–10 m, depending on local site conditions) stress-relief measurements in the database. For stress-relief measurements made at depths >10 m (or at least one excavation diameter away from the free surface in a mine) the assigned data quality depends on the internal consistency of multiple measurements.

Global stress patterns

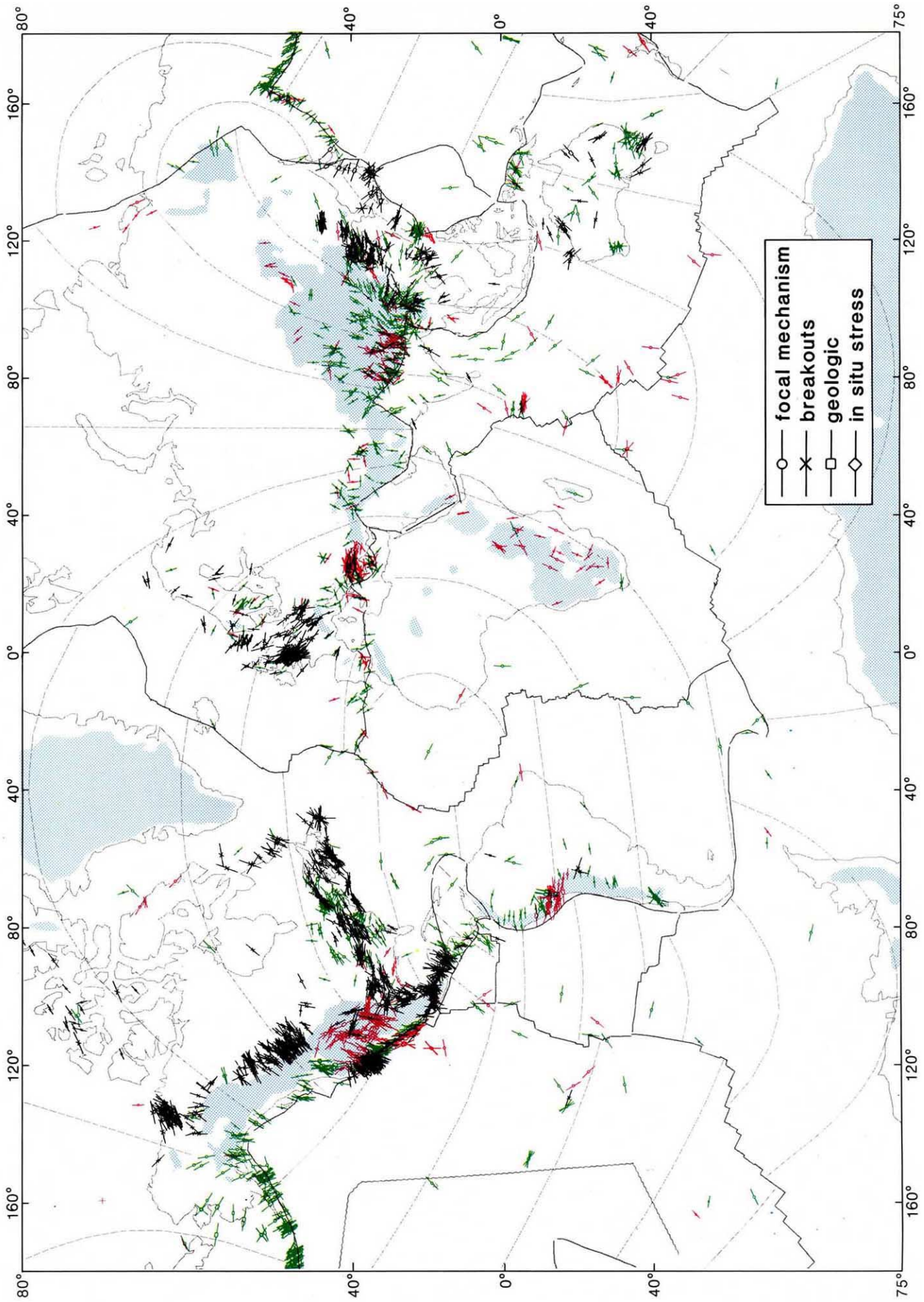
The first global map of stress orientations was published by Ranalli and Chandler in 1975⁶⁴ and contained 59 stress-relief measurements. In 1979, Richardson *et al.*¹⁹ presented a map containing about 133 points worldwide, derived primarily from earthquake focal mechanisms supplemented by surface overcoring measurements and geological data. Much of the data shown on these maps we would now categorize as rather low-quality (C or D) indicators of tectonic stress.

At present there are 3,574 entries in our global database; 3,142 of them are assigned either an A, B or C quality and are plotted on Fig. 2. As mentioned earlier, the emphasis in this paper is on the tectonic stress field, so D-quality data are not plotted. The length of each data point in Fig. 2 is proportional to its quality and the centre symbol designates the type of stress indicator. The distribution of the data by measurement type is as follows: earthquake focal mechanisms, 50%; breakouts, 31%; hydraulic fracturing measurements, 3%; stress relief, 7%; volcanic alignments, 4%; and fault-slip data, 5%. Distribution of areal coverage according to quality is given in Table 1.

A single horizontal stress orientation, S_{Hmax} , is plotted on Fig. 2 to facilitate the recognition of regional patterns of stress orientation. Relative stress magnitudes (stress regime) are indicated by colour for all data for which they are directly determined. Plotted in red are data that indicate an extensional stress regime (defined here as one in which the vertical stress is the

TABLE 1 Areal distribution of data

Area	A quality	B quality	C quality
N. American plate	450	635	555
USA	189	398	402
Canada	160	158	101
Mexico	93	69	28
Central America	8	10	24
S. American plate	7	5	23
Eurasian plate	100	388	577
Europe	66	83	181
Asia	34	305	396
Indo-Australia plate	16	74	99
Pacific plate	0	28	2
Africa plate	0	22	37
Antarctica plate	0	22	7
Nazca plate	0	9	0
Cocos plate	0	4	0



◀ FIG. 2 World stress map, transverse mercator projection. S_{Hmax} orientations are plotted, length of lines are proportional to quality (A, B or C). Because the data coverage is so dense in the western United States, only A- and B-quality data are plotted there. Centre symbol designates type of stress indicator. Data indicating an extensional stress regime are plotted in red, data for a compressional regime are plotted in green, and data where stress regime is unknown are plotted in black. All regions with average elevation greater than 1 km are indicated by blue shading. Lightly dashed lines correspond to absolute velocity trajectories determined using AM1–2 poles of Minster and Jordan⁶⁵. Wavy line approximates the 80-Myr isochron on the Pacific plate.

greatest principal stress); normal or combined normal and strike-slip faulting characterize this regime. The data in green indicate a compressional stress regime, in which one or both of the horizontal stresses are greater than the vertical stress; deformation in this regime is characterized by thrust, strike-slip or combined thrust and strike-slip faulting. Stress orientations for which information on relative stress magnitudes was not determined directly or not reported are plotted in black. Unfortunately this is the case for all of the breakout data, which comprise nearly one-third of the data set.

Regions with average elevation greater than 1,000 m are indicated on Fig. 2 by shading. Also shown are absolute plate-velocity trajectories, calculated using Minster and Jordan's⁶⁵ poles of angular motion for each plate (model AM1–2).

Three broad generalizations can be drawn from the data. (1) Most midplate regions are characterized by compressive stress regimes, that is, by thrust and/or a combination of thrust and strike-slip deformation. (2) Most continental areas of extensional stress regimes (normal or combined normal and strike-slip faulting) are regions of anomalously high elevation (for example, the western US Cordillera, the East African rift, the Baikal rift, the Himalayas and the Andes). (3) Broad regions of the Earth's crust are subjected to a nearly uniformly oriented stress field, specifically a $\sim 2 \times 10^7$ -km² region (approximately one-eighth of the world's land surface) of north-east to east-north-east compression in central and eastern North America and a $\sim 6 \times 10^6$ -km² region of north-west to north-north-west compression in western Europe.

In the midplate portions of some plates there is a striking correlation between S_{Hmax} orientations and azimuths of absolute plate velocity (as indicated by the trajectories in Fig. 2). This correlation is illustrated by the histograms in Fig. 3, which show, point by point, the angular difference between the observed S_{Hmax} orientation and the local absolute-velocity azimuth. In these plots the local absolute-plate-velocity direction is the reference direction; hence positive angles in Fig. 3 represent observed S_{Hmax} directions oriented clockwise with respect to that reference direction. It is important to note that because these histograms represent a point-by-point count and not a regionally averaged fit, they are strongly influenced by regions of dense data distribution. Furthermore, all data of A to C quality are treated equally, not weighted by quality.

Figure 3 indicates a strong positive correlation between S_{Hmax} orientations and absolute velocity directions on the two fastest-moving continental plates: midplate North America (including most of the central and the eastern United States, much of Canada and possibly the western Atlantic basin) and the South American plate. The correlation is particularly striking for midplate North America, the area of densest and highest-quality data coverage in the world; here the angular differences show a near-normal distribution about the reference absolute velocity direction. Breakouts on the outer continental shelf in eastern North America contribute to scatter in the histogram (see shaded areas on Fig. 3); detailed analysis of these data indicate that S_{Hmax} orientations are often rotated into a plane approximately parallel to the local trend of the continental slope. This rotation may be the result of superposition of stresses owing to flexure from sediment loading on the continental shelf⁶⁶.

The strong positive correlation between S_{Hmax} and absolute velocity directions for midplate North America has been interpreted by some workers as indicating that resistive drag at the base of the plate is the primary source of stress in the upper lithosphere⁶⁷. However, as pointed out previously, the absolute velocity vector in midplate North America coincides within $\sim 10^\circ$ with the expected direction of the ridge-push force from the Mid-Atlantic Ridge^{28,68–70}; therefore the two mechanisms cannot be distinguished as possible sources of stress for this plate. Similarly, for the South American plate, the approximately east-west absolute velocity is within 10° of the ridge-push direction inferred from the South American–African pole of relative motion. In addition, convergence between the South American plate and both the Nazca and Pacific plates is also approximately east-west. Thus, for both the North and South American plates it is not apparent which source of stress, if any, is dominant. Overall, the simplest explanation for the correlation between S_{Hmax} and absolute-plate-velocity directions on these plates is that the net balance of forces that move the plates dominates the stress distribution within the plate interiors.

In western Europe the S_{Hmax} directions and absolute-plate-velocity directions seem to be relatively well correlated (Fig. 2), except in the Aegean. However, as indicated in Fig. 3, the data consistently indicate a clockwise rotation of S_{Hmax} with respect to the absolute-velocity direction, that is, the observed S_{Hmax} orientations trend more northerly than the west-north-west absolute-velocity field. S_{Hmin} directions in the Aegean region (orthogonal to the S_{Hmax} directions shown in Fig. 3) are also clockwise with respect to the direction of plate motion. However, it should be noted that the absolute-velocity rotation pole for Eurasia is very poorly constrained because this plate is moving so slowly in an absolute reference frame⁶⁵. Thus, the significance of the difference between the S_{Hmax} and absolute-velocity direction is unclear.

Another potentially significant source of stress in Western Europe is the force resulting from the continental convergence of the African and Eurasian plates. Overall, S_{Hmax} orientations seem to be relatively well correlated with the predicted convergence velocity field obtained using the African–Eurasian pole of relative motion from the NUVEL velocity model⁷² (see histogram in the lowermost left corner of Fig. 3). S_{Hmax} orientations in the Aegean are generally orthogonal to the convergence direction (see below). However, the average S_{Hmax} direction of $N 42^\circ W$ obtained from the extensive breakout data set in the United Kingdom⁷¹ is more northerly than that predicted from the velocity field for this region on the basis of either the absolute-velocity directions ($N 62^\circ W$), ridge-push forces ($N 70^\circ W$), or continental-convergence forces ($N 56^\circ W$). To properly evaluate the stress field resulting from these plate-boundary forces, however, detailed two- or three-dimensional finite-element modelling is needed.

There is the suggestion of a weak correlation between S_{Hmax} and absolute-velocity directions in the Pacific plate, although the data for this plate are few in number and derived primarily from earthquake focal mechanisms. Data from older (north-western and western) portions of the Pacific plate have S_{Hmax} orientations that are generally orthogonal to absolute-velocity directions (Fig. 3). S_{Hmax} orientations in the younger portions of the Pacific plate are quite scattered with respect to absolute plate motion; however, if events near the East Pacific Rise are excluded, S_{Hmax} orientations in the younger portions of the plate are sub-parallel to the absolute plate-velocity direction (Fig. 2). Interestingly, this change in S_{Hmax} orientations coincides roughly with the 80-Myr isochron, the point at which the $t^{1/2}$ subsidence of cooling oceanic lithosphere dramatically slows down from $t^{1/2}$ to an approximately exponential decay⁷³. However, once again, spreading directions in the Pacific Plate, using relative motion poles with respect to both the Nazca and Antarctic plates, are aligned within 10° with absolute plate-motion vectors, so forces owing to ridge push and drag cannot

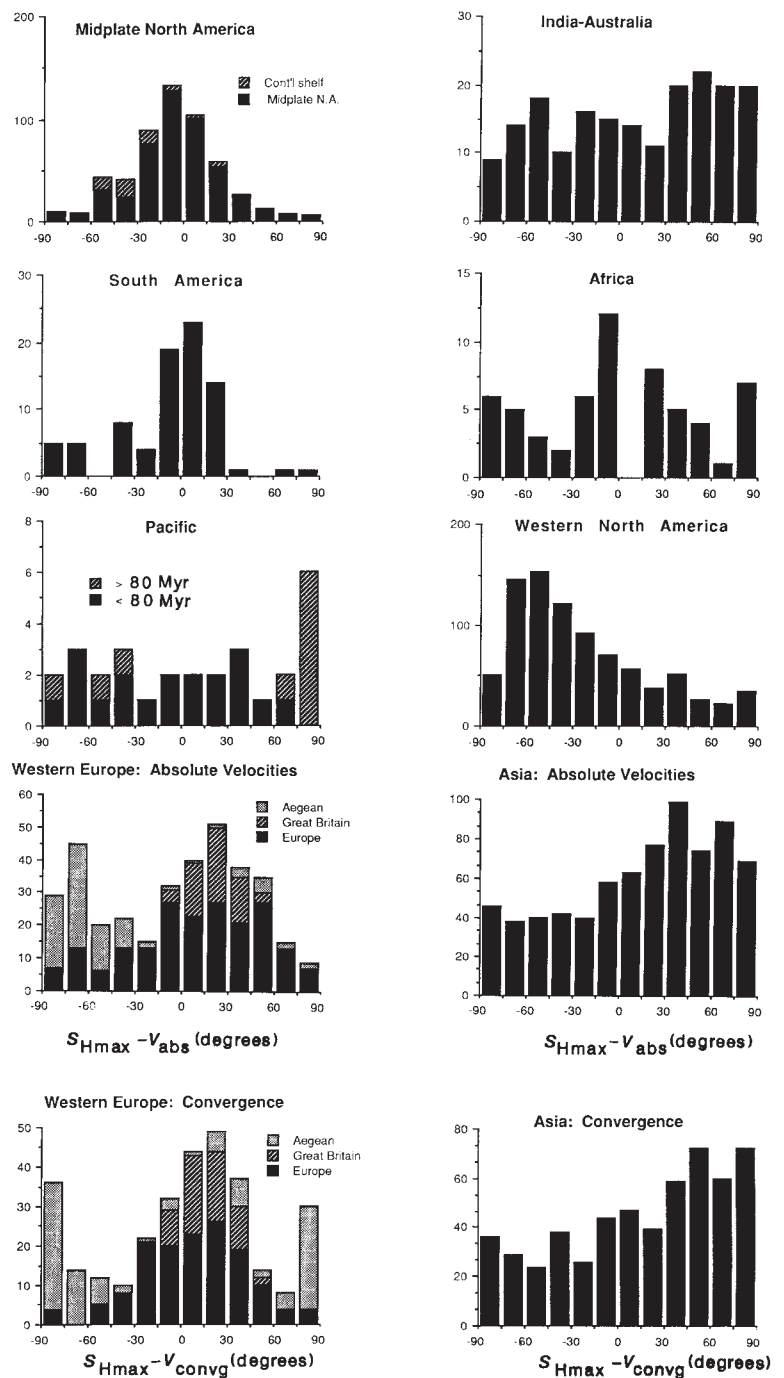


FIG. 3 Histograms comparing, point by point, the angular difference between observed S_{Hmax} orientations and a computed velocity field for specific plates, subplates or regions. Upper four histograms in both columns compare observed stress directions with absolute-velocity directions, and the lowest histogram in each column compares observed stress directions with relative (convergent) velocity directions (see text).

be distinguished. Although single-force finite-element models of resistive drag¹⁹ predict variations in relative stress magnitudes across the Pacific plate that are consistent with the observed stress rotation, it is impossible to determine the source of this apparent polarity change between S_{Hmax} and S_{hmin} without detailed modelling that includes all potential forces acting on the plate.

Figure 2 and the plots in Fig. 3 indicate that there is no clear correlation between S_{Hmax} and absolute velocity in several major regions, for example, much of Asia, the Indian-Australian plate, the African plate and the largely extensional and thermally uplifted western US Cordillera. Data for the Antarctic, Nazca and Cocos plates are too sparse either to confirm or to contradict any possible correlation.

The pattern of stress throughout eastern Asia is strongly influenced by the collision of the Eurasian and Indian-Australian plates. Within the Himalayan collision zone the S_{Hmax} orientations are generally north-south, subparallel to both the absolute motion of the Eurasian plate⁶⁵ and to the relative (convergent) motion between the Eurasian and Indian-Australian plates⁷². However, in eastern China, S_{Hmax} trajectories form a quasi-radial pattern which is oblique to both the absolute and the relative plate-motion directions (see histograms in the lower right corner of Fig. 3). This quasi-radial pattern of S_{Hmax} orientations is well predicted by indenter-type models⁷⁴ for the continued collision of the Eurasian and the Indian-Australian plates.

As indicated on Fig. 2, the pattern of S_{Hmax} orientations for

the Indian–Australian plate is rather complex; in particular, data for the Australian continent, a relatively stable intraplate region, show great variability (in contrast to stress patterns in midplate North America, for example). A high level of intraplate seismicity within the Indian Ocean has attracted a lot of interest in attempts to model the plate-driving and other tectonic forces acting on this plate^{75–77}. In these models, Himalayan collision generally accounts for the north-easterly compression in the eastern Indian Ocean, whereas complex intraplate stress patterns throughout the rest of the plate generally result from the complicated plate geometry and the varying nature of subduction along the Sunda and Tonga–Kermadec arcs.

Regions of extensional stress

As shown in Fig. 2, areas of extension on the continents occur primarily in regions of anomalously high elevation. These areas of extension occur in a variety of tectonic settings: a broad zone of distributed shear (western North America), an intraplate setting (Baikal and African rifts), a continental-collision zone (Himalayas), and above a subduction zone (Andes). The latter two examples are regions of relatively rapid plate convergence in which the extension direction is generally orthogonal to the convergence direction. In contrast, in true ‘back-arc spreading’, extension occurs in a direction generally orthogonal to the arc boundary, so that extension is often subparallel to convergence direction. Modern examples of back-arc spreading generally occur in topographically depressed areas such as the Marianas basin and other western Pacific basins (all of which are currently unsampled by the database). The modern low elevation of the Aegean region is best explained by Pliocene back-arc spreading which was subsequently replaced by modern (late Pleistocene to present) extension parallel to (that is, S_{Hmax} perpendicular to) the convergence direction⁷⁸ (see histogram in lower left corner of Fig. 3).

The relationship between elevation and extension is complex. For example, a recent analysis⁷⁹ indicates that extension in the high Tibetan Plateau cannot be explained by crustal thickening and elevated topography alone. For this extension to occur concomitant with continued collision and convergence, an additional force must be added to the system, such as a buoyancy force derived from a ‘deblobbing’ or delamination of thickened dense mantle lithosphere.

The contrast in stress patterns between the regionally high western US Cordillera and the ‘midplate’ region of North America suggests that the effects of Pacific–North American plate interaction are not transmitted through the western United States. The source of much of the uplift in the western United States must be thermal (or equivalently, thinned mantle lithosphere), as crustal thickness in much of this region is comparable to or less than that in the eastern United States⁸⁰. Details of the western United States stress pattern have been discussed elsewhere^{24,25,28,81}; however, it is of interest that throughout much of this elevated region the stress regime is extensional. In some cases, abrupt 90° changes in extension direction occur which appear to correlate with lateral changes in lithosphere thickness^{11,28} and thermal regime⁸².

Focal mechanisms within the African plate⁸³ show dominantly normal faulting within the thermally elevated East African rift system; events outside the rift have both strike-slip and normal faulting mechanisms. In southern Africa, overcoring measurements made in deep mines (up to 3 km depth)⁶³ and focal mechanisms suggest a distinct stress regime with a ~90° rotation of S_{Hmax} relative to the East African rift (Fig. 2). The general extensional stress state throughout much of the African plate is inconsistent with a compressional midplate stress field predicted solely from the plate-boundary forces (ridge push from ridges on three sides of the plate and a continental collision along the northern boundary)¹⁹. Furthermore, drag forces on this plate are probably minimal because Africa is moving so slowly in an absolute reference frame⁶⁵. The profound intraplate extension

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in East Africa suggests an additional source of stress, probably related to buoyancy associated with mantle upwelling and lithospheric thinning.

Conclusions and future work

The data and brief interpretations presented here represent a progress report intended to stimulate further interest and participation in the World Stress Map project. Analysis of the data compiled to date, together with the results of a large number of earlier regional studies, permit the following generalizations and conclusions:

- The various stress measurement and analysis techniques used in this study seem to sample contemporary regional stress orientations and show an excellent agreement despite the wide range of depths sampled.
- The state of stress throughout the upper, brittle crust is regionally consistent. This allows the definition of provinces of relatively constant orientations and relative magnitude of stress.
- Most intraplate or midplate regions are characterized by a compressional stress regime, that is, thrust or strike-slip faulting. Extensional stress regimes occur almost exclusively in anomalously high, and often thermally elevated regions, implying superposition of a buoyancy force fundamentally linked to the source of the elevation.
- A strong positive correlation between absolute plate velocity and S_{Hmax} direction for broad regions of several plates implies that the net plate-boundary forces responsible for moving these plates dominate the stress distribution in the plate interior. Evaluation of the relative importance of drag at the base of the plates will require detailed modelling constrained by data on absolute stress magnitudes.
- Local crustal structure, rheology and strength contrasts can strongly influence tectonic-stress orientations. Examples include stress rotation within a ~250-km-wide zone straddling the San Andreas fault and along continental margins.

We have several clear objectives for the future. First, we are currently cooperating with industry to obtain additional well-bore breakout data to fill in many of the gaps in the map; targeted areas include South America, Africa, the Middle East and south-east Asia. The oceans, which cover 70–75% of the Earth's surface, are greatly undersampled. Breakout analyses in two Deep Sea Drilling Project holes⁸⁴ confirmed the mean S_{Hmax} orientation inferred from nearby focal mechanisms, and established the feasibility of this stress-measurement technique in future Ocean Drilling Program holes. The oceanic data are essential for testing the preliminary observation of an approximately 90° rotation in S_{Hmax} between the younger and older portions of the Pacific plate and to constrain the magnitudes of

stresses acting on this, the fastest moving, plate.

Second, to understand fully the origins of lithospheric stresses, we must constrain both stress orientations and magnitudes. Hydraulic-fracturing stress-magnitude measurements in a limited number of deep holes (in both the continents and oceans) are clearly needed to understand the stress-boundary conditions on the plates as well as to constrain the stress levels required for intraplate deformation.

Once information on both stress orientation and magnitude becomes available, detailed modelling will be possible of the various sources of stress acting on different plates. Such models, when sufficiently constrained by data, should lead to a greatly improved understanding of plate-tectonic processes. □

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Cloning and structure of a yeast gene encoding a general transcription initiation factor TFIID that binds to the TATA box

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The TATA sequence-binding factor TFIID plays a central role both in promoter activation by RNA polymerase II and other common initiation factors, and in promoter regulation by gene-specific factors. The sequence of yeast TFIID, which seems to be encoded by a single gene, contains interesting structural motifs that are possibly involved in these functions, and is similar to sequences of bacterial sigma factors.

THE activation and regulation of promoters of protein-coding genes is mediated both by gene-specific factors that usually recognize specific DNA-sequence elements (see refs 1–3 for reviews), and by general initiation factors which interact with common core-promoter elements (see refs 4–8). The most common core-promoter element is the TATA sequence (or TATA box) (see refs 4 and 9) and an interacting transcription initiation factor, designated TFIID (ref. 10), has been identified in *Drosophila*¹¹, mammals^{4,12} and yeast^{13–15}. The binding of TFIID represents the first step in core-promoter activation⁴ and in turn facilitates stepwise interactions of RNA polymerase II and other general initiation factors^{5–8}. Consistent with its capacity for template commitment^{16–18}, TFIID seems to remain bound following initiation and promoter clearance⁵, and may catalyse multiple rounds of initiation.

In principle, any of the steps and factors involved in core-promoter activation are potential targets for the action of regulatory factors. Studies in yeast indicate that there are regulatory factor interactions with RNA polymerase II^{19,20}. Our previous studies (see ref. 15 for review) indicate that mammalian TFIID can be a direct target for both cellular and viral activators^{6,12,21–23}, and that stable binding of TFIID to the promoter can preclude repression mediated by chromatin assembly^{23,24}. Thus TFIID has a central role in transcriptional regulation because it both recruits other general factors to the promoter and serves as a target for regulatory factors. The importance of understanding the structure and function of TFIID has been emphasized by suggestions of the presence of functionally distinct TATA elements^{25,26}, which could be recognized by distinct factors.

The recent identification^{13,14} and complete purification¹⁵ of a yeast TATA box-binding protein that can substitute functionally for human TFIID provides an important breakthrough for investigations of the structure and function of TFIID in eukaryotes. Here we report the cloning and structural analysis of a yeast gene that encodes a functional TFIID.

Cloning of the gene that encodes TFIID

N-terminal amino-acid sequences were obtained from yeast TFIID purified by HPLC (ref. 15) and two cyanogen bromide (CNBr)-derived peptides. Sequence amplification of yeast DNA

mediated by the polymerase chain reaction (PCR), with primers based on these N-terminal sequences, yielded a single 656-base pair (bp) fragment, which contained the expected sequences in the same reading frame (Fig. 1). A yeast genomic library was screened with this fragment, and one of the positive clones (YCp2D) was found to contain an 8-kilobase pair (kb) DNA insert with all of the sequences encoding TFIID that were present in the subcloned PCR fragment. The sequence of a 1,140-bp segment containing these sequences (Fig. 1) showed a single long open reading frame (ORF) encoding a 240-amino-acid protein of calculated relative molecular mass (M_r) 26,986. This value compares favourably with M_r estimates of purified TFIID¹⁵ obtained from SDS-PAGE (M_r , 27,000) and Sephacryl S-300 gel filtration (M_r , 28,000). The region of YCp2D that was sequenced contained all of the natural TFIID-derived peptide sequences in the long ORF (arrows and underlined regions in Fig. 1), as well as a TATA box (data not shown) and an AATAAA polyadenylation signal (boxed in Fig. 1).

TFIID messenger RNA and chromosomal DNA

RNA blot analysis with the TFIID-DNA probe (Fig. 2a) revealed a discrete RNA species of ~1,100 nucleotides which was greatly enriched in the poly(A)-selected RNA and undetectable in the poly(A)⁻ fraction. Similarly, primer extension analysis (Fig. 2b) detected one main 5' end, which was ~190 bp upstream of the initiation methionine (boxed ATG in Fig. 1) and ~70 bp downstream of the TATA element (data not shown). Thus, TFIID mRNA consists of a single main species with a long 5' untranslated region. Although post-transcriptional control has been observed for other yeast mRNAs with long 5' untranslated regions²⁷, these RNAs, unlike TFIID mRNA, also contained short ORFs in the untranslated regions.

To investigate the copy number of the gene that encodes TFIID, genomic DNA was digested with each of three restriction endonucleases and blots of resolved fragments were probed with the PCR-derived insert from the plasmid vector pGEM7-2D (Fig. 2c). Consistent with the presence of only two *RsaI* sites in the coding sequence and no *EcoRI* and *HindIII* sites in the sequenced region of YCp2D (Fig. 1), digestion with these enzymes gave single hybridizing bands of 441 bp, ~2.5 kb, and ~7 kb, respectively (Fig. 2c). Although these analyses were performed under high-stringency hybridization conditions, similar analyses under low-stringency conditions failed to reveal additional bands. Altogether, these data indicate that there is only one gene that encodes the isolated TFIID; this gene has been mapped to chromosome 5 (D. Poon, unpublished results). Preliminary results from gene-disruption experiments (data not shown) indicate that this gene is essential.

In vitro expression of a functional TFIID

Sequences extending from nucleotides -223 to +723 relative to the translation start site (Fig. 1) on the YCp2D insert were cloned into the plasmid vector pRS315, and the resulting plasmid (pRS315-2D) was transcribed by phage T7 RNA polymerase.

Translation of these RNAs in a reticulocyte lysate led to the production (Fig. 3a) of a polypeptide with an M_r of 27,000 (p27), equivalent in size to purified TFIID¹⁵. Mobility shift assays with these same lysates also showed site-specific binding of the translated p27 to the adenovirus major late promoter; the complex that was formed had the same mobility as that generated with purified TFIID, and showed the same relative sensitivity to oligonucleotide competitors containing intact versus mutated TATA sequences (Fig. 3b). In addition, as previously reported for the natural yeast TFIID¹³⁻¹⁵, the expressed p27 could substitute for human TFIID in stimulating transcription from the adenovirus major late promoter. As shown in Fig. 3c, lysates programmed with pRS315-2D transcripts complemented endogenous TFIID-depleted HeLa-cell extracts (lanes 12-15), as did human TFIID (lanes 2-3), whereas lysates programmed with no RNA (lanes 4-7) or with bromo mosaic virus RNA (lanes 8-11) showed no TFIID activity. These results provided convincing evidence that the cloned YCp2D insert encoded a polypeptide of M_r 27,000 with TATA box-binding- and transcription initiation activities (TFIID).

Sequence alignments and structural motifs

Analysis of the sequence of the gene that encodes TFIID (Fig. 1) failed to show any of the highly conserved motifs (zinc fingers, helix-turn-helix motifs, or leucine zippers) that are characteristic of many other sequence-specific DNA-binding proteins (see ref. 3 for a review). Computer searches of the

EMBL and GENBANK data bases also failed to detect any genes with extensive sequence similarities to the gene for TFIID. One of the many proteins with weak sequence similarities to TFIID was the adenovirus E1A single-stranded DNA-binding protein²⁸ (23% identity and 37% similarity over 132 residues); this is consistent with the single-stranded DNA-binding activity of TFIID⁴ and the likelihood of promoter melting during initiation²⁹.

Given the functional similarities between TFIID and bacterial sigma factors²⁹, which include the ability to recognize TATA or TATA-like promoter elements, we made comparisons of their corresponding sequences (Fig. 4a). The sequence alignments were most extensive for the *Bacillus subtilis* phage SPO1-specific σ -factor spoIIAC (27% identity and 47% similarity over 30 residues), and less extensive for the primary sigma factors σ^{70} and σ^{43} (23% identity and 40% similarity over the same region). For each σ -factor, the region of greatest similarity contained part of the σ -factor 2.3 region (positions 1-18 in Fig. 4a), which has been suggested to be involved in single-stranded DNA binding²⁹, and all of the σ -factor 2.4 region (positions 19-42), implicated directly in interactions with the -10 consensus element (TATAAT consensus) of prokaryotic promoters²⁹⁻³¹. Like the σ -factor 2.3 regions²³, the TFIID region between residues 167 and 196 contains a high density of aromatic (and some basic) residues. Additionally, we have noted alternative sequence alignments between two regions that follow the aromatic-rich region of TFIID and part of the σ -factor 2.4 region

FIG. 1 Nucleotide sequence of the *S. cerevisiae* gene that encodes TFIID. Amino-acid sequences matching those determined for the N-terminal regions of natural TFIID and CNBr-derived polypeptides (I and II) are underlined. Primer DNA sequences used in the PCR cloning are marked by arrows. Translation start and stop codons, methionine residues N-terminal to CNBr-I and -II, and potential polyadenylation signal sequences are in boxes. Single nucleotides in boxes (residues 30 and 669) denote positions that differ in the PCR-derived clone; whether this reflects mismatch hybridization of degenerate primers or sequence variations in different yeast strains used for DNA cloning is unknown, but the variations do not affect the amino-acid sequence.

METHODS. From 30 μ g of an SP-Sepharose fraction¹⁵, reverse-phase HPLC-purified TFIID and two derived CNBr polypeptides (I and II) were obtained and sequenced as previously described^{15,36}. These sequences in the single-letter amino-acid code, with uncertain residues indicated by X or in parentheses, were: TFIID, NH₂-ADEERLKEFKENKIVFDNPTQVXENQ(R)-(D)(G)(T/I)-COOH; CNBr-I, NH₂-VVTGAKSEDDSKLASRYARIQX(K/I)-(G)F(S/A)(A/K)(D/K)(F)(T)(D)(F)(K)(I/S/R)(Q)(N)(I)(V)G-COOH; CNBr-II, NH₂-VKPKIVLLIFVSGKIVLTGAKQREIYQAF(E)(A)(I)(Y)(P)(V)-COOH. For the PCR, the sense primer based on TFIID N-terminal residues 7-12 (KEFKEA) was 5'-ATATGAATTCAA(A/G)GA(A/G)TT(C/T)AA(A/G)GA(A/G)GC-3' and the anti-sense primer based on CNBr-II residues 24-29 (EEIYQAF) was 3'-CT(C/T)CT(C/T)TA(A/G/T)AT(A/G)GT(C/T)CGTTGAAATTA-5'. The initial 10 nucleotides (underlined) in each oligonucleotide were included to generate restriction-endonuclease cleavage sites, although they were not used subsequently. Each primer (1 μ g) and either 0.1 or 1.0 μ g of either *S. cerevisiae* genomic DNA or total DNA of the YCp-based genomic library³⁷ template was boiled for 5 min and cooled slowly to 30 °C. After addition of nucleoside triphosphates (0.2 mM) and Taq polymerase (2.5 u) the reaction was subjected to cycles of 1 min at 94 °C, 2 min at 55 °C and 3 min at 72 °C in a Perkin-Elmer machine. After 50 cycles, the temperature was gradually decreased over 20 min to 24 °C and the final sample stored at 4 °C. Under these conditions the size and amount of the amplified DNA was independent of the DNA source or the amount of template DNA, and generated a single fragment that was cloned into pGEM7. Sequence analysis of the resulting plasmid (pGEM7-2D) showed a 656-bp insert with TFIID N-terminal residues 7-33, CNBr-I residues 1-41, and CNBr-II residues 1-29 in the same reading frame. The excised insert was labelled by nick translation and used to screen about 100,000 bacterial colonies from a YCp-based genomic library³⁷ by standard techniques. After hybridization, filters were subjected to three 10-min washes at room temperature in 2 $\times$ SSC, 0.1% SDS, and to one 15-min wash at 60 °C in 0.2 $\times$ SSC and 0.1% SDS before autoradiography. Two additional screens by the same procedure yielded 10 positive clones. The partial sequence of the clone (YCp2D) with the largest insert (~8 kb) is shown.

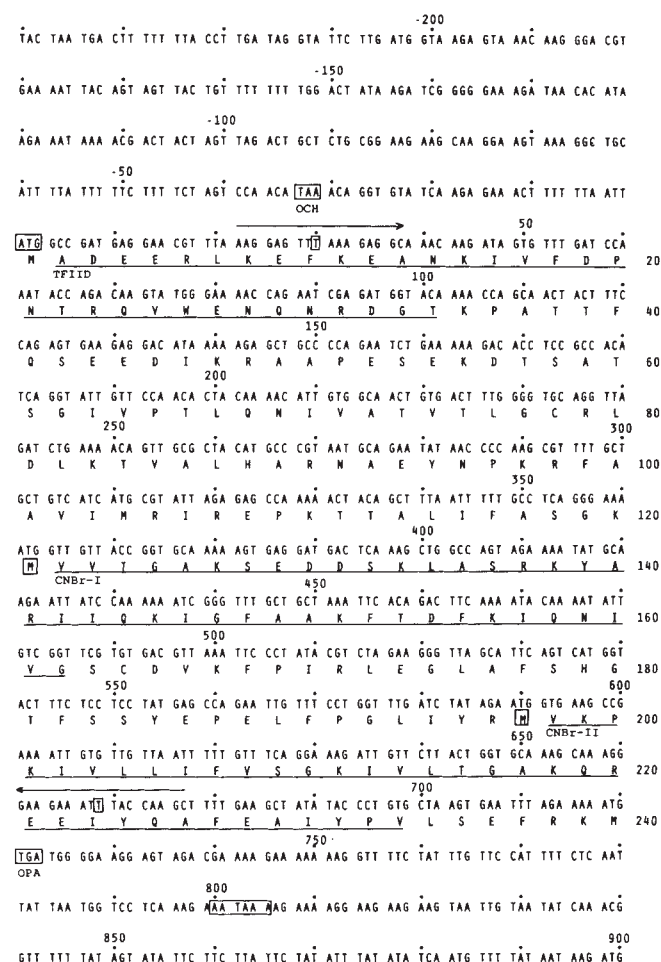
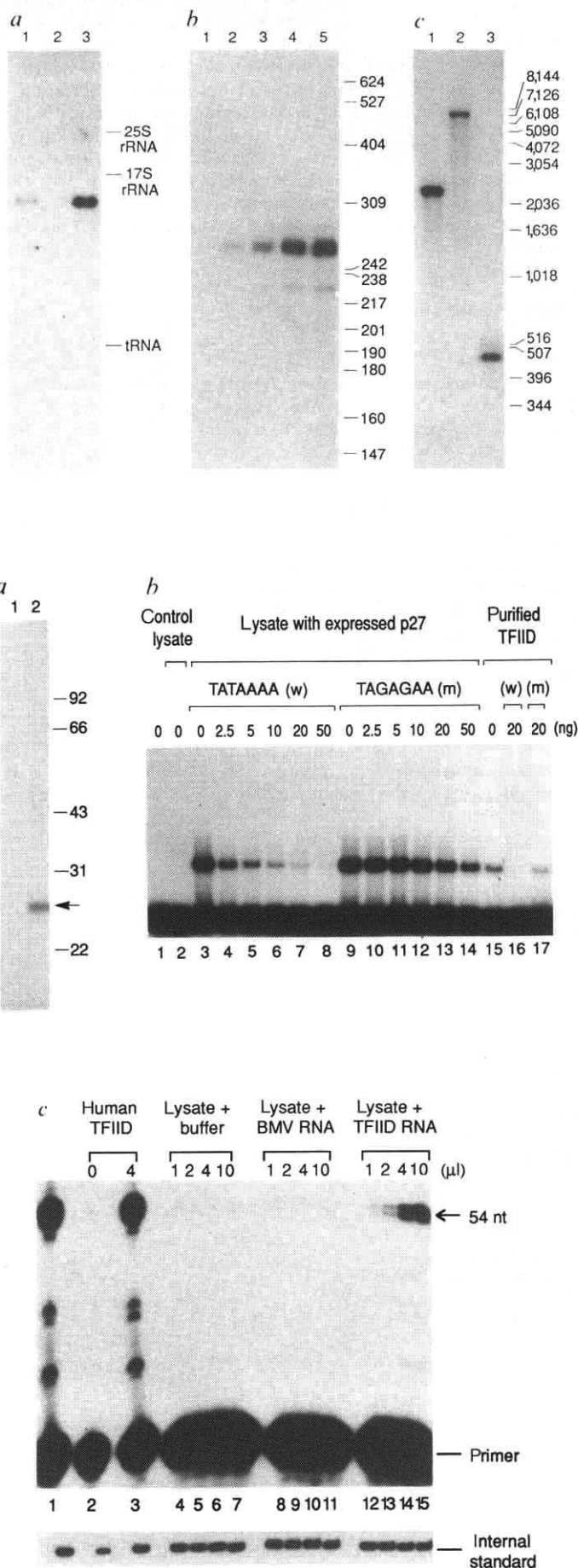


FIG. 2 Mapping of mRNA and genomic DNA. *a*, Northern blot of 20 μ g total RNA (lane 1), poly(A)⁻ RNA (lane 2) and poly(A)⁺ RNA (lane 3). The positions of size markers (~3,700, 2,200 and 100 nt) are indicated. *b*, Primer extension analysis of poly(A)⁺ RNA (0, 2, 5, 10 and 20 μ g in lanes 1–5) with size markers indicated. *c*, Southern blot analysis of genomic DNA digested with *Eco*RI, *Hind*III and *Rsa*I (lanes 1–3) with size markers shown.

METHODS. Total RNA from *S. cerevisiae* strain BJ926 (grown to late log phase in yeast-extract peptone dextrose) was fractionated into poly(A)⁻ and poly(A)⁺ fractions by oligo(dT)-cellulose chromatography. For the northern analysis (*a*), RNA samples were fractionated on 1.5% agarose gels containing 2 M formaldehyde, transferred to nitrocellulose paper (Schleicher and Schuell BA85), UV-crosslinked and probed with a random primer-labelled *Hind*III insert (nucleotides 122–1616) from pGEM7-2D. Blots were washed twice for 30 min at 65 °C in 2 × SSPE–0.1% SDS, and twice for 15 min at 65 °C in 0.2 × SSPE–0.1% SDS before autoradiography. Ethidium bromide staining of the agarose gel indicated complete transfer of 17S- and smaller RNAs. Primer-extension mapping of the 5' end (*b*) used a 5' end-labelled oligonucleotide complementary to nucleotides +34 to +63 (Fig. 1), and resolution on a standard 10% polyacrylamide-urea sequencing gel before autoradiography. For the Southern blot (*c*), 1 μ g samples of genomic DNA were digested with the indicated enzymes, fractionated in 0.8% (w/v) agarose gels, depurinated, transferred to nitrocellulose membranes and probed with labelled fragments used for the northern blot. Blots were subjected to four 10-min washes in 2 × SSC and 0.1% SDS at 20 °C, and four 30-min washes in 0.1 × SSC and 0.1% SDS at 50 °C before autoradiography. Unless otherwise noted, standard procedures were used. Size markers were yeast ribosomal (r)- and transfer (t) RNAs (*a*), *Hpa*II-digested pBR322 fragments (*b*) and BRL 1-kb markers (*c*).

FIG. 3 Expression and function of cloned yeast TFIIID. *a*, SDS-PAGE analysis of [³⁵S]methionine-labelled TFIIID expressed in a reticulocyte lysate. Lysates were programmed with no RNA (lane 1) or cloned TFIIID-derived mRNA (lane 2). *b*, Site-specific DNA binding of expressed yeast TFIIID. Electrophoretic mobility shift assays with an adenovirus major late promoter (MLP) fragment contained: lane 1, no protein; lane 2, lysate programmed with brome mosaic virus (BMV) RNA (negative control); lanes 3–14, lysate programmed with TFIIID mRNA; lanes 15–17, purified yeast TFIIID. Competing oligonucleotides (MLP; positions –45 to –15) contained either wild type (w)- or mutant (m) TATA sequences (as indicated at the top) and were added in the nanogram amounts indicated at the top of each lane. The arrow indicates the specific protein-DNA complex. *c*, Transcription activation by expressed TFIIID. Transcription from the adenovirus MLP was measured in a complete HeLa-cell nuclear extract (lane 1) or in a TFIIID-depleted nuclear extract⁴ complemented with (1) buffer only (lane 2), (2) human TFIIID (lane 3) or (3) increasing amounts (1, 2, 4 and 10 μ l) of reticulocyte lysate programmed with buffer (lanes 4–7) or BMV RNA (lanes 8–11) or RNA transcribed from the cloned yeast TFIIID gene (lanes 12–15). Specific transcripts are indicated by the 54-nucleotide (nt) band.

METHODS. A phage T7 RNA polymerase-driven expression plasmid (pRS315-2D) containing sequences of the gene for TFIIID from –223 to +723 was constructed as follows. We synthesized one oligonucleotide (5'-GGCCGGCCGAGCTCACCTTGATAGGTATTCTTGA-3') with sequences (underlined) complementary to 5' sequences of the gene for TFIIID and with a *Sac*I recognition site (GAGCTC) 9 nt from the 5' end. A second oligonucleotide (5'-GGCCGGCCGCTGTCAGTTACTATCACATTTTCTAAATTCAC-3') was synthesized, with sequences (underlined) complementary to 3' sequences of the gene for TFIIID and with a *Pst*I recognition site (CTGCAG) 10 nucleotides from the 5' end followed immediately by two additional in-frame translation stop signals (UAA and UAG). These two oligonucleotides were used to amplify sequences of the gene encoding TFIIID using the PCR conditions described in Fig. 1. The *Sac*I–*Pst*I fragment was cloned into pRS315³⁸ by means of the *Sac*I and *Pst*I sites, and one plasmid (pRS315-2D) was sequenced to verify that the coding region and translation signals were intact. The plasmid was transcribed with phage T7 RNA polymerase and amounts (1 μ g) of purified RNA used to template reticulocyte lysates (Promega). Translation products were either analysed by SDS-PAGE (after [³⁵S] methionine labelling) (*a*) or employed for site-specific binding (*b*) and functional (*c*) assays. Mobility shift assays used either 0.5 μ l lysate or 0.4 ng purified yeast TFIIID and an end-labelled fragment extending from positions –138 to +46 of the Ad MLP. Conditions were as previously described¹⁵, except that reactions also contained 20 ng poly(dG–dC). Transcription assays used untreated HeLa-cell nuclear extracts or HeLa-cell extracts in which endogenous TFIIID was inactivated by a 15-min incubation at 47 °C (ref. 4), an intact plasmid template (pSmaF) containing the adenovirus MLP, and either partially purified human TFIIID⁴ or reticulocyte lysate programmed with TFIIID mRNA. Specifically initiated adenovirus MLP transcripts were monitored by reverse-transcriptase extension of a 30-nucleotide primer complementary to nucleotides +25 to +54.



(Fig. 4a, bottom). These alignments emphasize highly conserved hydrophobic residues at every fourth position, followed by position-specific basic residues³¹.

Yeast TFIID has a net positive charge ($pI=9.84$) with a central region (Fig. 4b) rich in basic (especially lysine) residues. The central part of the proline-free region between residues 110 and 168 has a high potential for α -helix formation (data not shown), and the entire array of basic residues in this region could be on the same face of an α -helix (Fig. 4c, d). Both the central core of five basic residues and the repeat pattern of some of the lysines (at 7- and 11-, or at 18-residue intervals) may be important for this region. Possible functions for this region are discussed below.

Discussion

In this report we have described the cloning of a yeast gene that encodes TFIID. This gene is transcribed to give a discrete unspliced mRNA. Our confirmation that active yeast TFIID is a monomeric (apparently unprocessed) protein of M_r 27,000 leads to the suggestion that the functionally interchangeable human factor, which appears to be much larger^{32,33} and binds to an extended region on the promoter⁴, is either heteromeric or consists of a larger polypeptide possibly possessing additional functional domains (for example, regulatory domains). Related to this point is whether the yeast factor is capable of mediating all the regulatory factor interactions that are mediated by human TFIID. Our recent studies (with E. Sinn, unpublished results) have indicated that yeast TFIID can mediate herpesvirus (pseudorabies) IE-dependent transcription *in vitro*.

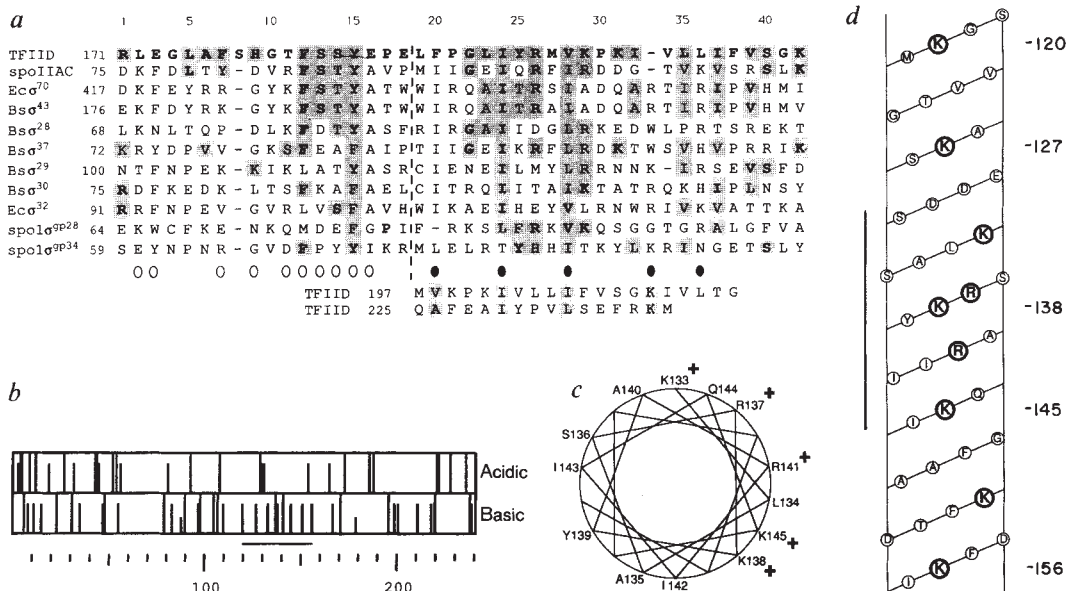
The central role of TFIID in promoter activation and regulation necessitates several separate or overlapping structural domains, which include those for DNA binding, interactions with basic factors, and interactions with regulatory factors. Therefore it might be expected that there is an overall TFIID structure that is distinct from, and less flexible than, those of the DNA-sequence-specific activators described so far. Consistent with this is the failure of computer searches to reveal structural motifs in TFIID that are commonly found in many

other DNA-binding domains, or strong sequence similarities (homologies) between TFIID and other proteins involved in nucleic acid function.

More direct sequence comparisons have shown weak sequence similarities of regions of TFIID with conserved regions of bacterial σ -factors. This is of interest because there are general functional similarities between TFIID and σ -factors in directing core-promoter recognition by corresponding RNA polymerases (along with other factors in eukaryotes), and because similar sequence elements are recognized during these processes. Thus TFIID interacts most strongly with an element (consensus sequence TATAAA) that resembles the common -10 consensus sequence TATAAT of many bacterial promoters. The general similarity between a region of TFIID and the aromatic rich σ -factor 2.3 region is of potential interest, because the σ -factor may recognize single-stranded DNA when the -10 consensus is melted in the bacterial pre-initiation complex²⁹ and because of expectations that eukaryotic TATA-initiation-site regions may also be melted during initiation. The more extensive similarities between TFIID and σ -factor 2.4 regions are interesting because of the genetic evidence for direct contacts between the σ -factor 2.4 region and the -10 promoter region. Internal deletions in the σ -factor-related region of TFIID eliminate the ability of TFIID to bind to the TATA box (T. Yamamoto, unpublished results), consistent with this region being involved in promoter binding. Future studies will determine whether the sequence similarities are of significance and whether any of the other general functions of σ -factors are retained in TFIID. Although highly conserved regions of σ -factors have been implicated in core RNA polymerase interactions²⁹, the observation⁵⁻⁷ that promoter recognition by RNA polymerase II requires factors in addition to TFIID indicates that TFIID may not contain a similar domain.

Also of interest is the potential distribution in TFIID, along one face of an α -helix, of a central cluster of basic residues that includes a short Arg-Lys repeat and a longer Lys repeat. The probability of this region being involved in DNA recognition is raised by the recent implication of basic domains in such

FIG. 4 Structural features of yeast TFIID. *a*, Alignment of TFIID sequence with bacterial σ -factor sequences (single-letter amino-acid code). *Escherichia coli* (Ec), *B. subtilis* (Bs) and bacteriophage SPO1 (spo1) σ -factor sequences are from refs 29 and 31. The position in the protein sequence of the first residue in each line is indicated in the second column. Amino acids identical to those in TFIID are in bold and conserved amino acids (relative to the TFIID sequence) are shaded according to the groupings (K, R, H), (E, D), (N, Q), (G, P), (A, I, L, V), (F, W, Y), (M, C), and (S, T). The open circles indicate the conserved positions in the σ -factor 2.3 domain (left of dashed line), whereas the solid circles denote only those positions in the σ -factor 2.4 domain (right of dashed line) that are most highly conserved³¹ in all the σ -factors. The shaded areas in the lower two lines show those TFIID residues that match the most conserved σ -factor 2.4 region residues. *b*, Positions of acidic and basic residues in TFIID. Short bars, D or K, tall bars, E or R. *c*, Helical wheel depiction of TFIID residues 133-145. *d*, Flattened helix display of TFIID residues



118-157 (region underlined in *b*), with basic residues in bold. The helix was flattened (see ref. 34) by splitting it lengthwise along the face opposite to the aligned lysines at positions 120, 127, 138, 145 and 156. The split residues at positions 136 and 154 are shown in duplicate. The vertical bar on the left side indicates the region depicted in *c*.

functions³⁴. Alternatively this region could interact with specific domains in other transcription factors, such as the acidic domains of upstream activators (see ref. 2 for a review) or the phosphorylated C-terminal repeat of the largest RNA polymerase II subunit (see ref. 35 for a review). The amphipathic nature of some activating domains², such as those in GAL4 and GAL4 derivatives, make this interaction model more likely; indeed a direct effect of GAL4 derivatives on mammalian TFIID-promoter interactions has been demonstrated²¹. The presence in TFIID of repeated structures might also allow simultaneous, synergistic interactions of multiple activators². Alternatively, activators could also interact with other components of the basic transcription machinery.

Given functionally distinct σ -factors in prokaryotes²⁹ and reports of functionally distinct TATA elements in eukaryotes^{25,26}, the idea of multiple TATA-binding factors in

eukaryotes is appealing. Although we detected only a single essential gene that encodes the predominant form of TFIID in *Saccharomyces cerevisiae*, our studies do not eliminate the possibility of multiple, perhaps more distantly related, genes that encode factors with similar functions. Could TFIID be thus involved in the transcription from promoters that do not have TATA elements? Human TFIID can interact not only with the TATA element but also with DNA sequences around and downstream of the initiation site^{4,12}, with other general factors⁵⁻⁷ and with upstream factors^{6,12,21}. The combined effects of these interactions could therefore serve to stabilize TFIID in a pre-initiation complex, and allow its normal function, even in the absence of strong TATA element interactions. The availability of a homogenous yeast TFIID, along with the appropriate genetic analyses in yeast, should allow these ideas to be tested. □

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Expression of two myogenic regulatory factors myogenin and MyoD1 during mouse embryogenesis

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MyoD1 and myogenin are muscle-specific proteins which can convert non-myogenic cells in culture to differentiated muscle fibres, implicating them in myogenic determination. The pattern of expression of MyoD1 and myogenin during the early stages of muscle formation in the mouse embryo *in vivo* and in limb-bud explants cultured *in vitro*, indicates that they may have different functions in different types of muscle during development.

MYOGENIC cell lines and primary cultures of myoblasts from muscle tissue undergo many of the steps characteristic of ter-

минаl myogenic differentiation *in vivo*. These steps include cell fusion to form multinucleated myotubes and the accumulation of muscle-specific transcripts and their products¹. Thus, the transition of myoblasts to myotubes provides a useful system for the study of myogenesis, in particular the regulation of genes that encode muscle structural proteins and enzymes.

Until recently, regulatory steps which precede terminal differentiation have been less accessible to investigation. We have previously isolated two complementary DNA sequences for MyoD1^{2,3} and myogenin⁴, which seem to have a role in myogenic determination and differentiation; non-muscle cells transfected with these sequences can be converted to the myogenic pathway. Data obtained from cultured muscle-cell lines indicate that MyoD1, which may be constitutively expressed in myoblasts, should appear earlier during develop-

ment than myogenin, which is expressed during myoblast terminal differentiation. Northern blot analysis of isolated tissues has shown that the transcripts for MyoD1 and myogenin are specific to skeletal muscle during late fetal, newborn and adult stages^{2,4}. We have now examined the expression of these two transcripts during embryogenesis to ascertain whether myogenesis *in vivo* involves the same factors as those isolated from cell lines.

We localized the myogenin and MyoD1 transcripts *in situ* in tissue sections of mouse embryos and in limb bud explants cultivated *in vitro*. Myogenin transcripts were first detected in the myotomal cells of the first somites, whereas MyoD1 transcripts began to accumulate ~2 days later. By contrast, MyoD1 and myogenin transcripts became detectable during limb development at the same time. These observations indicate that

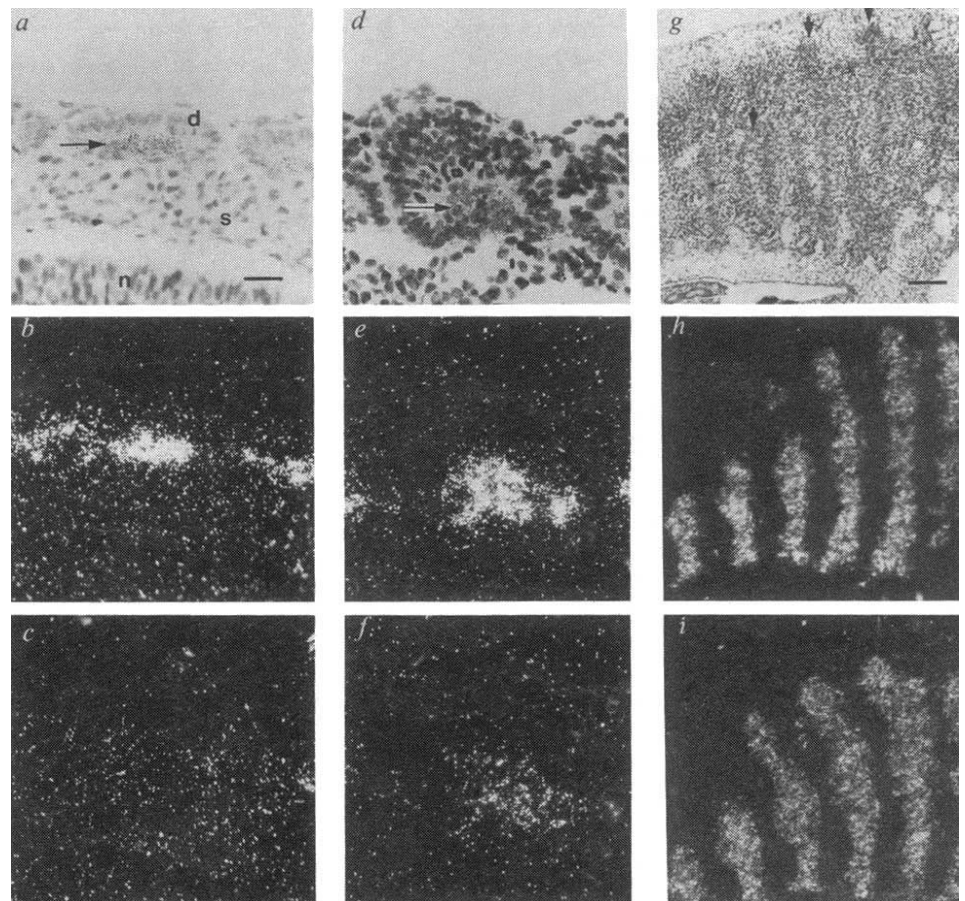
distinct populations of myogenic precursor cells exist in the embryo, which differ in their pattern of expression of MyoD1 and myogenin. Although all the cells present in the limb bud explant were initially negative for myogenin and MyoD1, positive cells subsequently appeared and the initial stages of muscle formation took place as *in vivo*. Because all limb musculature in vertebrates is probably derived from cells in the somites, these experiments indicate that an earlier myogenic precursor cell is present, which does not contain detectable levels of myogenin or MyoD1.

Myogenin and MyoD1 expression in the somites

Myogenin transcripts were first detected at ~8.5 days *post coitum* (p.c.) in the myotome compartment of the somites, and were expressed at high levels by 9.25 days p.c. (Fig. 1*a, b*). The onset

FIG. 1 *a-c*, Expression of myogenin and MyoD1 in somites of a 9.25-day-p.c. (24 somites) mouse embryo. *a*, Photomicrograph of two caudal somites from a 9.25-day-p.c. mouse embryo, cut sagittally. This section was hybridized with a cRNA probe for myogenin mRNA and even under light-field optics intense silver-grain accumulation was observed over the myotomal (indicated by an arrow) by a compartment S sclerotome; d, dermatome; n, neural tube. *b*, Darkfield photomicrograph of section shown in *a*. Close-to-saturating grain densities were observed for myogenin. At this stage, detection of myogenin was restricted to the somites, predominantly over the myotomal portion. *c*, Darkfield photomicrograph of a section immediately adjacent to that in *a* and *b*. This section was hybridized with a probe for MyoD1 mRNA. No accumulation of grains was detected, indicating that transcripts for MyoD1 were either absent or present in very low quantities. Section thickness, 1.0 μm . *d-f*, Expression of myogenin and MyoD1 in somites of a 10.5-day-p.c. mouse embryo. *d*, Photomicrograph of rostral somites. This section has been hybridized with a cRNA probe for myogenin mRNA, and silver-grain accumulation was observed over the myotomal compartment (arrow). *e*, Darkfield photomicrograph of section in *d*. At this stage, detection of myogenin was still restricted to the somites. *f*, Darkfield photomicrograph of a section immediately adjacent to that in *d* and *e*. This section was hybridized with a probe for MyoD1 mRNA. A light accumulation of grains was detected indicating that transcripts for MyoD1 were present. Section thickness, 5–7 μm . *g-i*, Expression of myogenin and MyoD1 in myotomes of an 11.5-day-p.c. mouse embryo. *g*, Lightfield photomicrograph of rostral myotomes showing muscle masses (arrows) in between sclerotomal (precostal) tissue. *h*, Same section as in *g*, viewed with darkfield optics showing myogenin expression in myotomal masses. *i*, Adjacent section viewed with darkfield optics hybridized with MyoD1. Labelling is more dispersed but of equal intensity to that for myogenin at this stage. Section thickness, 5–7 μm . Scale bars: *a-f*, 50 μm ; *g-i*, 150 μm .

METHODS. Preparation of probes: A cDNA corresponding to myogenin was subcloned into a Bluescribe M13⁺ (Vector cloning Systems). The cRNA transcripts were synthesized according to manufacturer's conditions and labelled with ³⁵S-labelled UTP (>1000 Ci mmol⁻¹; NEN). Linearization with *Pst*I followed by transcription with T7, yielded a cRNA corresponding to the terminal 700 nucleotides of the cDNA, which is specific for myogenin transcripts and which does not contain regions homologous to MyoD1 transcripts, including the *c-myc*-like region⁴. The cRNA transcripts were subjected to alkali hydrolysis⁵ to give a mean size of 60 bases for efficient hybridization. A cDNA corresponding to MyoD1 was subcloned into a Henikoff-



modified Bluescribe vector and linearized with *Mlu*I to give an antisense probe corresponding to the 3' part of the cDNA, from nucleotides 751 to 1,785 (see ref. 2). Radiolabelled transcripts were made as for myogenin. Again, the site of linearization was chosen to yield a cRNA specific for MyoD1 transcripts and which did not contain domains corresponding to the *c-myc* region. For both probes, the final concentration was adjusted to 90,000 d.p.m. μl^{-1} in hybridization buffer. Results were directly compared with our previous results using cRNA probes to the α -actins from the same embryos on adjacent tissue sections⁵. In addition, sibling or age-matched embryos were studied to ensure that all somites were represented. Cells were considered labelled if grain density was ≥ 5 times that of non-muscle cells. Because non-muscle cells had background levels rarely exceeding 2 grains per cell, these cells were considered as unlabelled. Procedures used for embryo preparation and subsequent histological treatment were as described previously⁵. Sections of 1 μm were obtained, by cutting cooled paraffin blocks of tissue in a cryostat (Bright Instruments), equipped with a motor drive, at -18°C . Sections were collected on subbed slides and relaxed on a drop of distilled water-1% ethanol solution, and then treated as regular tissue sections. Embryos used for these studies were C3H and C3H $\times$ BALB/c. Embryonic development was monitored according to ref. 22. For summary of results see Table 1.

TABLE 1 Expression of myogenin and MyoD1 in mouse somites and limb buds

Embryo age	Rostral somites				Caudal somites				Limb buds (fore and hind)		
	Myogenin	MyoD1	Cardiac α -actin	MHC	Myogenin	MyoD1	Cardiac α -actin	MHC	Myogenin	MyoD1	Cardiac α -actin
8.5 days p.c. (2)	++	—	+	—							
9 days p.c. (2)	+++	—	+	—					—	—	—
9.25 days p.c. (2)	+++	—	++	—	+++	—	++	—	—	—	—
9.5 days p.c. (2)	+++	—	++	+	+++	—	++	—	—	—	—
10.5 days p.c. (4)	+++	+	++	++	+++	+	++	—	—	—	—
11.5 days p.c. (5)	+++	+++	+++	+++	+++	+++	+++	+++	++	++	++

Results are summarized from *in situ* hybridization experiments on mouse embryos with probes for myogenin, MyoD1, cardiac α -actin, and myosin heavy chain (MHC) sequences. Probes for myogenin and MyoD1 transcripts were as described in Fig. 1. Transcripts for cardiac α -actin were detected with a 5' non-coding sequence probe specific for this messenger RNA⁵. MHC transcripts were detected with a coding-sequence probe derived from the 3' end of the β -MHC mRNA (150 base pairs (bp), *Pst*I–*Pst*I fragment), which cross-hybridizes with the different striated-muscle MHC mRNAs present (G.L., manuscript in preparation). Hybridizations were performed on the numbers of embryos indicated in parentheses. Paraffin sections were 5–7 μ m thick, except for embryos at 9.25 days which were sectioned at 1.0 μ m. In general, three serial sections from two different embryos (that is, six sections) were hybridized together on the same slide with one probe. The next three sections were hybridized with a second probe, and so on. Hybridization was performed alternately with different probes to be certain that a negative result with one probe was flanked by positive results with the other, and thus was not due to the plane of section. The numbers of sections generated ranged from 50–100 per embryo. An indication of relative intensities is given; this is based on visual impression, however, and represents only an approximation of the relative levels of transcripts. The intensities reflect both density of labelling over a single cell and the number of positive cells. No indication is given here of the regional distribution of transcripts in the somite or limb bud, for example between myotomal cells and myocytes in the somite⁵. MHC expression is only seen in differentiated myocytes of the myotomal muscle. The morning of vaginal plug was counted as post-coital (p.c.) day 0.5; cardiac development and numbers of somites were followed as a further check on dating, and corrections were made on this basis. As there is an anterior–posterior developmental gradient, data for 'rostral' somites are for the most anterior somites, and 'caudal' for the most posterior somites.

of myogenin expression in the somites at 8.5 days p.c. coincided with that of cardiac α -actin and preceded detectable myosin expression (see Table 1), which implied that full differentiation to functional muscle had not occurred by then^{1,5}. By contrast, detailed analysis of almost all somites present in these embryos failed to detect any significant MyoD1 signal. Two embryos (each 9.25 days p.c.; 24 somites) were serially sectioned at 1 μ m, and sections were alternately hybridized with probes for either myogenin or MyoD1. In all cases where the myotomal compartment was morphologically discernible, intensely labelled myogenin transcripts were easily detectable; no significant labelling over background was observed with MyoD1, however, either in the somites (Fig. 1c) or elsewhere in the embryo, even after prolonged exposure times (11–14 days versus 7 days). Because of the thinness of the sections (1 μ m) and the fact that almost all of the sections that were negative for MyoD1 were immediately adjacent to sections that were positive for myogenin, it was unlikely that adjacent sections did not represent comparable zones in any given somite. These results indicate that transcripts for MyoD1 are either absent or are present at very low levels in cells expressing both cardiac α -actin⁵ and myogenin.

By 10.5 days p.c., the myotomes of the somites differentiate into segmented blocks of myotomal muscle^{5–7}. We observed myogenin labelling throughout the myotomal compartment (Fig. 1d, e). At this stage MyoD1 began to be detectable over the somites and muscle myotomes (Fig. 1f). Because somitogenesis occurs in a rostral–caudal direction, myotomes first appeared at the anterior portion of the embryo, whereas less mature somites which lacked myotomal muscle were present near the tail. Both the anterior myotomal muscle and the posterior somites at this stage were positive for MyoD1. Thus, no obvious morphological change was evident that coincided with the appearance of MyoD1. By contrast, muscle myosins were expressed at this time only where myotomal muscle was forming in the rostral myotomes (see Table 1). It has been proposed⁸ that an induction, possibly neural, occurs in all the somites by 10.5 days, which changes the myogenic programme of somite-derived myoblasts. The accumulation of MyoD1 transcripts correlates with this phenomenon, which may represent a change in the myogenic programme.

By 11.5 days p.c., intense labelling (both of myogenin and MyoD1) is still observed over all somite segments, including

the most posterior somite. In addition, intercostal, intervertebral and various other muscle anlagen are highly positive for both probes. At 15 days p.c., all muscle masses maintain high levels of myogenin and MyoD1. The relatively 'late' appearance of MyoD1 indicates that the earliest myogenesis in the embryo (myotomal muscle) may begin in the absence of this factor. It is therefore unlikely that MyoD1 is a trigger for these early events, although its eventual presence indicates that it plays a part during subsequent stages of myotomal development. The appearance of different muscle-marker sequences that we examined during myotomal muscle formation is summarized in Table 1.

No expression of myogenin or MyoD1 was detected in non-skeletal muscle tissue in the post-implantation embryos that we examined. The heart, from the early stages of cardiac-tube formation (7.5 days) onwards, showed no expression, despite the fact that many of the contractile protein genes that are expressed in developing skeletal muscle are also expressed in cardiac muscle. This observation (see also refs 2, 3 and 4) clearly indicates that neither myogenin nor MyoD1 is necessarily a prerequisite for sarcomeric gene expression. Thus, although these proteins may activate downstream myogenic structural genes, they are strictly specific for skeletal myogenesis.

Myogenin and MyoD1 expression in the limb

Even though the forelimbs are readily distinguishable as 'limb buds' at 9 days p.c., we first detected myogenin and MyoD1 transcripts at 11.5 days p.c. in the proximal region of both the hindlimb and the forelimb. In contrast to the situation in the somites, there was no detectable asynchrony in the accumulation of the two transcripts (Fig. 2 and Table 1). At this stage, myogenin and MyoD1 were expressed in mononucleate cells, in limb tissue devoid of muscle fibres. At later stages of limb development, both the transcripts accumulated in the differentiated muscle masses (results not shown), as they did in myotomal muscle (Fig. 1g, h, i).

In several systems it has been demonstrated that the myoblasts that invade the developing limb bud originate from the somite (derma-myotome)^{9–14}. In the mouse, a significant proportion of the mesodermal cells that will eventually contribute to the limb musculature are presumably present in the limb bud at ~9 days p.c.¹⁴. We tested whether the lack of detectable MyoD1-positive myoblasts in the limb bud before 11.5 days p.c. was due to an

absence of myogenic cells by removing limb buds from mouse embryos at 9.25 days p.c. (~24-somite stage) and placing them in organ culture. Such explants attach to the bottom of the culture dish in ~24 h and cells then begin to migrate out of the explant tissue. Transcripts for MyoD1 were not detectable in the limb bud or any other part of the intact embryo at the time of limb removal (9.25 days p.c.). After 4 and 5 days in culture, limb buds were analysed by *in situ* hybridization. We found strong labelling for both MyoD1 and myogenin in proximal regions of the explant (Fig. 3). These experiments indicate that the precursor cells for muscle are present in the limb and initially show no detectable expression of MyoD1 and myogenin.

Discussion

Our results clearly demonstrate that the pattern of expression of myogenin and MyoD1 in the precursor cells for myotomal muscle differs from that in the precursor cells for the musculature of the limb. Whereas MyoD1 transcripts were first detected ~2 days later than the first detection of myogenin transcripts in myotomal muscle, both transcripts appeared at the same time in the limb. This not only implies that MyoD1 and myogenin may be used differently at different developmental stages, but also indicates the existence of different populations of precursor muscle cells.

The experiments with the explanted limb bud indicate that at 9.25 days myogenic precursor cells are present which do not express either MyoD1 or myogenin. It is possible that undetectable levels of MyoD1 are present in the 9.25-day-p.c. limb and have a significant myogenic role here or in the somites. If so, the levels of transcripts are markedly lower than those seen later

in development. It is also possible that cells which leave the somite and invade the limb bud are not yet determined for the myogenic lineage and thus do not express MyoD1 or myogenin. But embryological experiments indicate that all skeletal muscle in the limb is derived from the somites, and that the somites contribute to no other limb tissue⁹⁻¹². This would mean that the cells that migrate from somites are already determined for myogenesis.

Somites present at the level of the limbs in the chick-quail chimaera system and in the mouse begin to send out cells to the limb bud just before the stage when the limb bud is discernable as a discrete structure¹⁰. Cells originating from a transplanted somite can be readily observed at the level of the limb bud as early as 20 h after transplantation¹². Although the number of such cells is initially small, we would expect there to be enough to be included in the sections examined and therefore to be observed should they express either MyoD1 or myogenin. We favour the hypothesis that in the limb bud there are determined myoblasts which have not begun to express MyoD1 or myogenin. This would imply that either the cells in the somite from which they derive are negative for MyoD1 and myogenin, and that those cells that are positive in the somite are committed to myotomal rather than limb-muscle formation, or that there are cells which transiently express these factors and then cease expression as they migrate away from the somite, perhaps because of subsequent cell proliferation.

The sequences of the genes encoding MyoD1 and myogenin share a region of homology, and both are entirely specific for skeletal muscle. The genes encoding MyoD1 and myogenin are probably part of a larger gene family of regulatory factors

FIG. 2 Expression of MyoD1 and myogenin in the forelimb bud of a 11.5-day-p.c. embryo. *a*, Lightfield photomicrograph. Section thickness, 5–7 μ m. *b*, Darkfield image of the same section hybridized with a cRNA probe for MyoD1 showing intense labelling to muscle anlagen. *c*, Darkfield image of an adjacent section hybridized with a cRNA probe for myogenin showing intense labelling to muscle anlagen. For Methods, see legend to Fig. 1 and Table 1. Scale bar, 100 μ m.

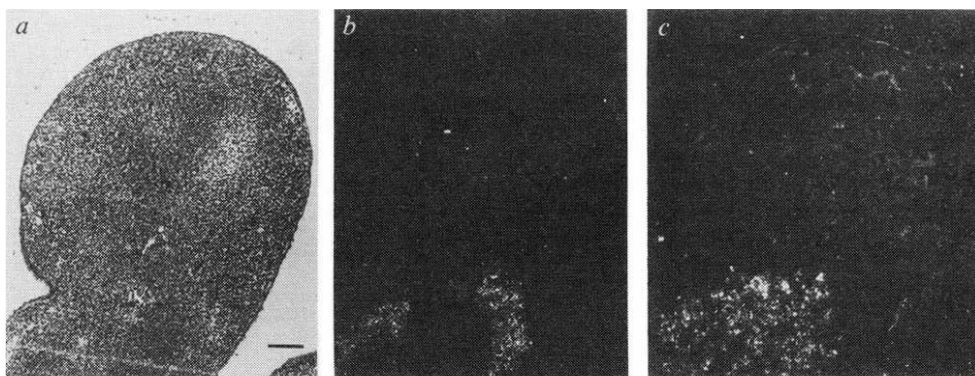
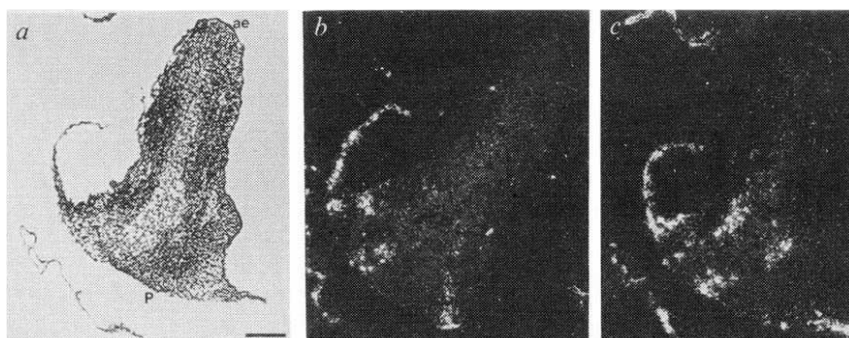


FIG. 3 Expression of myogenin and MyoD1 in a cultured embryo limb bud removed before any detectable accumulation of MyoD1 transcripts. *a*, Lightfield photomicrograph of cultured limb bud showing apical ectoderm (ae) and former proximal portion of limb (P) which was attached to culture dish. *b*, Darkfield image of an adjacent section hybridized with a cRNA probe for myogenin, showing labelling in the more proximal region of the limb bud. *c*, Darkfield image of the section in *a* hybridized with a cRNA probe for MyoD1, showing labelling which is also in the more proximal region of the cultured limb bud. Scale bar, 100 μ m.



METHODS. *In vitro* limb-bud explants: 9.25-day-p.c. embryos (24 somites) were removed from CO₂-anaesthetized female mice and placed in fresh, cold phosphate-buffered saline (0.13 M NaCl, 7 mM Na₂PO₄, pH 7.4). Somites were counted under a dissection microscope (Wild) and forelimb buds removed using glass and tungsten needles. The limb buds were then transferred to a 22 mm plastic culture dish (Falcon) in Dulbecco's-modified Eagle's medium (Gibco) supplemented with 10% FCS (Gibco) and 3% chick embryonic extract. Limb buds normally became anchored within 24 h, at which point the medium was

replaced daily until fixation and histological preparation for *in situ* hybridization. Limb 'outgrowths' were removed from the dish with a pipette after overnight fixation in fresh 4% paraformaldehyde in phosphate-buffered saline. All subsequent steps were identical to those previously described (ref. 5; also Fig. 1 legend). At least 15-limb-bud explants were examined. Those shown were cultivated for 4 days.

involved in myogenesis; these factors may have overlapping specificities for activating downstream structural and regulatory genes for terminal differentiation. Indeed, another sequence of this kind, *myf-5*, has been isolated from human muscle tissue¹⁵, and a potential myogenic determination factor, *Myd*, has also been described¹⁶. It has been speculated that different classes of myoblast exist throughout normal development, based on the observation that myoblast populations can be isolated which have a different behaviour in culture or express different myosin

types (that is, fast versus slow myosin heavy chains¹⁷⁻²¹). Our observation that at least two different modes of expression exist for myogenin and MyoD1, which coincide with two different types of muscle in the embryo (earlier somite myotomal muscle compared with later-stage myotubes in the limb), indicates that these factors may have different roles in conferring muscle-type determination and differentiation during different stages of embryogenesis. □

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LETTERS TO NATURE

Polarized light in high-redshift radio galaxies

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HIGH-redshift radio galaxies are characterized by ultraviolet excesses^{1,2} associated with elongated continuum and emission-line structures aligned along their radio axes^{3,4}. Commonly, these properties are interpreted in terms of jet-induced star formation. However, observations of a blue, polarized continuum knot in the low-redshift radio galaxy PKS2152-69 have suggested an alternative beaming/scattering model^{5,6}. To test this model we have obtained imaging-polarimetry observations of two high-redshift radio galaxies: 3C277.2 ($z=0.766$) and 3C368 ($z=1.132$). We find that both sources are strongly linearly polarized at ultraviolet rest wavelengths, with E-vectors within 25° of perpendicular to the axis of the extended continuum and radio structures. This implies that a large proportion of the ultraviolet light in these objects does not come directly from stars and we suggest it is scattered radiation from the nucleus. These observations also have important implications for beaming models of the active nuclei and for our understanding of the evolution of the host galaxies.

Ultraviolet excesses are an ubiquitous feature of high-redshift radio galaxies^{1,2}. Attempts to understand this excess have centred on star formation models, although it has been difficult to explain the ultraviolet-optical and optical-infrared colours simultaneously in the framework of galaxy evolution models². Recent clues to the nature of the ultraviolet excess have come from observations that show that they are associated with elongated or multi-modal continuum structures aligned along the radio axes^{3,4,7,8}. This link between radio and ultraviolet structures has led to the idea that some of the star formation in the host galaxies is connected with the radio jet, perhaps induced by the passage of the jet through the interstellar medium^{3,9,10}. The details of this mechanism are not well understood, however, and there is little observational evidence for such processes at low redshifts. In fact, out of the several dozen

low-redshift radio galaxies that have been studied in detail only NGC541 (Minkowski's object)¹¹ and perhaps NGC7385¹² show any evidence of star formation associated with radio jets, and these are much lower radio power sources than those observed at high redshifts.

An alternative beaming/scattering mechanism has been suggested⁵ based on observations of a high ionization cloud close to the radio axis of the low-redshift ($z=0.0282$) radio galaxy PKS2152-69. These reveal that a continuum source within the cloud, after subtraction of the smooth stellar light from the galaxy, has both an extremely blue spectrum ($f_\nu \propto \nu^{+(3\pm 1)}$) and a large linear polarization ($12 \pm 3\%$), properties that seem most consistent with a model in which the extended continuum is formed by scattering from dust of an intense beam of ultraviolet/optical radiation from the nucleus⁵. The importance of these observations to the high-redshift objects is that, if we were to observe PKS2152-69 in the ultraviolet, it would appear to have very similar properties to those observed in the high-redshift galaxies, albeit with a lower luminosity. This has led us to propose that the elongated continuum and emission-line structures in the high-redshift objects are somewhat analogous to reflection nebulae: at least a fraction of the extended continuum represents light scattered out of the intense blazar beam by material in the cloud, whereas the emission-line regions are ionized by Lyman continuum radiation from the beam⁶.

To test the beaming/scattering model we have begun a programme of imaging polarimetry of high-redshift radio galaxies using the ESO Faint Object Spectrograph and Camera (EFOSC) on the ESO 3.6-m telescope at La Silla, Chile. Using a Wollaston prism, EFOSC projects on a CCD (charge-coupled device) two perpendicularly polarized images of the field separated by 20 arcsec. A mask in the telescope focal plane with alternating opaque and transparent strips prevents object confusion and overlapping sky signal at the expense of half the field. A change of the instrumental position angle ϕ by rotating the whole EFOSC allows variation of the direction of polarization of the image pair. Further details of our imaging-polarimetry technique are given elsewhere¹³ (see also Fig. 1 legend).

The two objects observed (3C277.2 and 3C368) were selected on the basis that they were known to have closely aligned radio/optical/ultraviolet structures³ and are accessible from La Silla. For 3C277.2 we obtained three images at two different position angles through a B (0.44 μm) filter, whereas for 3C368 we have six images all at different position angles through a V (0.55 μm) filter and five images at two position angles through

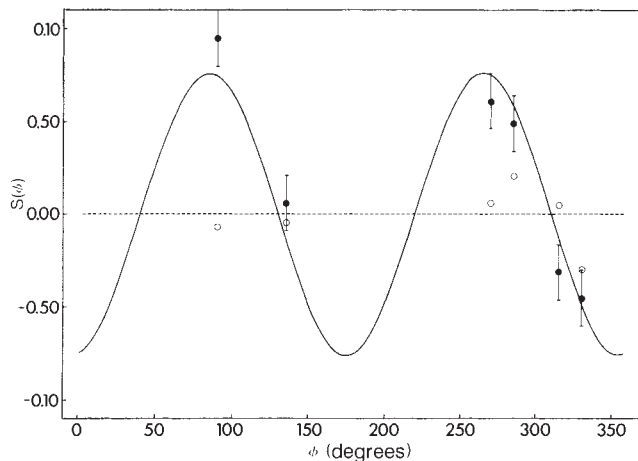


FIG. 1 Measurements (●) of $S(\phi)$, the component of the normalized Stokes parameters for linear polarization, for the V images of 3C368 and the fitted cosine curve from the formula given below. Also shown is the average $S(\phi)$ for faint stars in the same frames (○). For each frame recorded at instrumental position angle ϕ a parameter $S(\phi)$ is derived from the intensities of the two images of the object $i(\phi)$ and $i(\phi + 90^\circ)$ and from the average intensities of bright-field stars, which are assumed to be unpolarized, $i^u(\phi)$ and $i^u(\phi + 90^\circ)$:

$$S(\phi) = \left(\frac{i(\phi)/i(\phi + 90^\circ)}{i^u(\phi)/i^u(\phi + 90^\circ)} - 1 \right) / \left(\frac{i(\phi)/i(\phi + 90^\circ)}{i^u(\phi)/i^u(\phi + 90^\circ)} + 1 \right) \\ = P \cos 2(\theta - \phi)$$

where P is the degree of linear polarization and θ is the position angle of the plane of vibration of the $\mathbf{E}$ vector. P and θ can then be derived from two or more observations taken at different position angles¹³.

an R (0.70 μm) filter. The results are presented in Table 1. To make a preliminary check on the spatial distribution of polarization across 3C368, which is clearly resolved, we have measured that the polarization of the nucleus alone is not higher than the integrated polarization. The extended parts of the galaxy must therefore be polarized. A similar check is not possible for 3C277.2, because its bright central component, which we could measure, is only slightly extended in the direction of the radio axis.

To gain a reliable understanding of the errors inherent in this type of observation, we have submitted the data to several tests, which are described below. First, we emphasize that each value of $S(\phi)$ is derived from measurements made on a single frame and that effects of instrumental polarization (usually below 1%) are already removed in $S(\phi)$ using bright stars assumed to be unpolarized. For 3C368 in the V band, where we have images at more than two position angles, all the measurements of $S(\phi)$ are consistent with the expected cosine curve (see Fig. 1).

Because the main difficulty in the measurement of the intensity

of the various source images is the sky subtraction, the errors on P and θ are derived from the standard deviation in the measurement of the sky over object-free apertures of the same size as those used for the galaxies, propagated through the calculation of $S(\phi)$ and, subsequently, the fitting procedure using Monte-Carlo simulations. To check whether any systematic non-linearity is present in the polarization measurement, which is not removed by the correction for instrumental polarization because this uses stars that are much brighter than the galaxies, we have measured the average $S(\phi)$ for four stars in each V frame of 3C368, the brightnesses of which bracket that of the galaxy. The results are shown in Fig. 1.

We have also examined the possibility that a fraction of the observed polarization might be induced by the interstellar medium in the Galaxy. Using the relationship¹⁴ between the maximum amount of galactic interstellar polarization (P_{ISM}) and the galactic extinction¹⁵ we find that $P_{\text{ISM}} \leq 0.05\%$ for 3C277.2 ($b = 79^\circ$) and $P_{\text{ISM}} \leq 1.2\%$ for 3C368 ($b = 15^\circ$). For 3C368 the position angle of the $\mathbf{E}$ vector of the polarization induced by the interstellar medium of our galaxy would be close to the one measured for the object¹⁶. We have therefore looked at the polarization of an extended object at 43 arcsec in position angle 338° from 3C368, which has the image profile of a galaxy. The component of the polarization of this object along θ of 3C368 is below 0.5% in the V band. The null polarization measured for 3C277.2B provides a similar argument, although the very low galactic extinction already excludes any contribution of galactic interstellar polarization for 3C277.2.

Finally we estimate that the contribution of emission lines is $\sim 9\%$ of the detected radiation in both filters for 3C368, using the equivalent widths of Djorgovski *et al.*¹⁷. If the lines are unpolarized, then for the continuum alone $P_{\text{corr}} = 8.3 \pm 0.9\%$ in V and $P_{\text{corr}} = 2.7 \pm 1.2\%$ in R.

The polarization measured for 3C277.2 is larger than that measured in the optical for any extended, lobe-dominated radio source at low redshift¹⁸ and is at the upper end of the range observed in the highly polarized, violently variable quasars¹⁹. The V-band polarization for 3C368, although somewhat lower, is still larger than that measured for the nuclei of most low-redshift extended radio sources. In this source we also have evidence that the polarization is wavelength dependent, as the R-band measurements give a lower polarization.

The strongest conclusion from our results is that a large proportion of the ultraviolet light in the two galaxies is non-stellar. This property, especially if it proves to be a general one, has important implications for models that seek to explain the ultraviolet excess in terms of star formation, jet induced or otherwise. It is unlikely that the integrated light from the two sources is dominated by highly polarized quasar-like sources in the nuclei because such sources are invariably associated with compact radio structures and small emission-line equivalent widths, whereas both 3C368 and 3C277.2 have extended, lobe-dominated radio sources^{17,20} and 3C368 at least has large emission-line equivalent widths¹⁷. One possibility is that there

TABLE 1 Polarimetry results for high-redshift galaxies

Object	z	Obs. date	Filter	$\Delta\lambda_{\text{rest}}$ (Å)	Magn.	P (%)	P_{corr} (%)	θ (deg)	PA_{rad} (deg)
3C277.2	0.766	10 Apr. 1989	B	2150–2850	B=22.0	21 ± 4	21 ± 4	164 ± 6	61
3C277.2B	—	10 Apr. 1989	B	—	B=22.5	6 ± 7	0 ± 7	—	—
3C368	1.132	18 July 1988	V	2250–3000	V=21.4	7.6 ± 0.9	7.6 ± 0.9	85 ± 4	18
3C368	1.132	19 July 1988	R	2650–3750	R=20.5	2.8 ± 1.2	2.5 ± 1.2	92 ± 15	18

Results refer to the integrated light from each source. 3C277.2B is an extended object at 7 arcsec to the north-east from 3C277.2 and was labelled galaxy B by Laing *et al.*²⁷ P_{corr} is the degree of linear polarization corrected for the bias expected in the definite positive quantity P for low P/σ_P ²⁸. $\Delta\lambda_{\text{rest}}$ is the rest frame wavelength range covered by the observation and PA_{rad} is the position angle of the radio axis³.

is a contribution to the polarized light from synchrotron radiation emitted by the radio plasma, although this seems improbable given the great differences between the ultraviolet and radio morphologies.

The alternative hypothesis, the scattering of beamed radiation from the galactic nucleus, is consistent with the data. The small misalignments ($<25^\circ$) between θ and the perpendicular to the radio/optical axis can be explained by a plausible geometry for the light beam and for the scatterers. We do not exclude that some of the light might come from stars especially in the rest-frame V band. An indication that this might be true comes from the recent observation that the elongation and alignment with the radio axis are present also in the K band ($2.2\ \mu\text{m}$) in at least a couple of cases^{21,22}. Further observations in this band are necessary to show if there are differences with the morphology in the optical bands and to establish the importance of the stellar component. The beaming and scattering hypothesis requires that the nuclei of these galaxies emit intense collimated beams of ultraviolet/X-ray radiation and is therefore relevant to the 'unified schemes'^{23,24} for the active nuclei. As a scattering medium, both electrons in a hot halo^{25,26} and circumgalactic dust⁶ remain viable alternatives and can, in principle, be distinguished from wavelength dependences. For 3C368, our observation that the polarized flux per unit frequency in the V band is about twice that in the R band favours dust scattering, unless the beamed nuclear radiation is much bluer than observed in blazars. A strong test of the scattering mechanism is the observation of the spatial variation of the direction of the **E** vector which should always remain perpendicular to the direction from the light source for a single scattering process. Such observations will be difficult to make on these faint sources and measurements of the direction of the **E** vector in integrated light, although indicative, do not constitute such a definite criterion. Whichever proves to be the dominant scattering mechanism, polarization measurements covering a wide wavelength range will be important in the diagnosis of the nature and evolution of the interstellar medium and close environments of these active galaxies at early epochs. $\square$

The hot interstellar medium toward SN1987A

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AN X-RAY image of the field surrounding the supernova SN1987A, derived from the Einstein Observatory Large Magellanic Cloud (LMC) Survey, has allowed us to constrain the properties of the hot ionized medium along the line of sight to the supernova. If the absorption feature observed in the optical spectrum of the supernova at $6,379.7\ \text{\AA}^{1,2}$ is identified with $[\text{Fe X}] \lambda = 6,375\ \text{\AA}$, we find that an extraordinarily high iron abundance must be present, either in the hot interstellar medium of the LMC or in coronal gas between that galaxy and the Milky Way.

SN1987A is located on the outskirts of the 30 Doradus region, a large star-formation complex that was observed extensively with the imaging proportional counter (IPC) on board the Einstein Observatory (see ref. 3 for a description of the instrument and the Observatory). In Fig. 1, we present a 0.15–3.5 keV X-ray image of the region surrounding the site of the supernova; the data have been background subtracted, and the instrument response has been deconvolved using a maximum-entropy algorithm (Q.W. and D.J.H., manuscript in preparation).

This field includes several large OB associations⁴ (groups of massive main-sequence stars) and diffuse nebular emission in both the optical and radio bands^{5,6}. The X-ray surface brightness⁷ displays a morphology similar to that of the optical filaments, which are sketched in Fig. 1 for reference. There is no indication that the ISM at the position of the supernova differs significantly from that in the surrounding region. Thus, we would expect that the line of sight to SN1987A samples an ISM with the same general properties as that in the surrounding regions, unless there is a local ($<10\ \text{pc}$) variation associated with SN1987A itself.

We have obtained the X-ray surface brightness in the direction of the supernova by summing the flux in a circle of 2-arcmin radius centred at right ascension (1950) = $05^{\text{h}} 35^{\text{m}} 50^{\text{s}}$, declination (1950) = $-69^\circ 17' 58''$: $S_{\text{X}}(0.15\text{--}3.5\ \text{keV}) \leq 2.4 \times 10^{-3}\ \text{photons s}^{-1}\ \text{arcmin}^{-2}$. Adopting a spectral model⁸ for an optically thin thermal plasma at a temperature of $10^6\ \text{K}$, the temperature at which the equilibrium ionization fraction of Fe^{+9} is at a maximum, we derive an X-ray surface emissivity of

$$L \leq 10^{34} \delta\ \text{erg s}^{-1}\ \text{arcmin}^{-2} \quad (1)$$

where δ is a dimensionless interstellar absorption correction and a distance of 50 kpc for the LMC has been adopted. (The location of the X-ray emitting region with respect to the observed neutral gas along this line of sight is unknown. Comparison of the X-ray surface brightness in this location with positions a few degrees away (off the LMC) suggests that the bulk of the emission is associated with the LMC implying a lower limit for the absorption equal to the galactic H I (neutral, predominantly atomic hydrogen) column density of $\sim 6 \times 10^{20}\ \text{cm}^{-2}$. An upper limit to the absorption would include the entire H I column density along this line of sight, $3.5 \times 10^{21}\ \text{cm}^{-2}$. With this range of possible values $25 \leq \delta \leq 600$.)

The hot ionized medium (HIM) X-ray volume emissivity ϵ in our observing band can be expressed as $\epsilon = n_e n_{\text{HIM}} \Lambda_{\text{R}}(T)\ \text{erg s}^{-1}\ \text{cm}^{-3}$ where n_e and n_{HIM} are the electron and ion number densities in the plasma and $\Lambda_{\text{R}}(T)$ is the cooling function $\Lambda_{\text{R}}(T) \approx 3 \times 10^{-23} \xi\ \text{erg cm}^3\ \text{s}^{-1}$, where we assume that the heavy elements all have their cosmic proportions with respect to the Fe abundance ξ in solar units. The function $\Lambda_{\text{R}}(T)$ is nearly constant over the temperature range $10^6\text{--}10^7\ \text{K}$. We can obtain a crude measure of the temperature of the X-ray emitting

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gas by comparing the ratio of detected counts in the hard (0.8–3.5 keV) and soft (0.15–0.8 keV) bands with model spectra that include the effects of the instrument response and various absorbing column densities. The observed hardness ratio in the 2-arcmin region surrounding the SN is 0.18 ± 0.08 —a range of temperatures from $0.5\text{--}1.5 \times 10^7$ K is consistent (at 90% confidence) with this value for an optically thin thermal plasma with solar abundances observed through the allowed range of absorbing column densities. Figure 2 shows the observed emissivity as a function of temperature for these different column densities.

We can now express the X-ray surface emissivity L as

$$7 \times 10^{16} n_e N_{\text{HIM}} \xi \text{ erg s}^{-1} \text{ arcmin}^{-2} \quad (2)$$

From equations (1) and (2), we obtain

$$n_e N_{\text{HIM}} \xi \leq 1.4 \times 10^{17} \delta \text{ cm}^{-5} \quad (3)$$

We wish to compare this result to the putative optical detection of coronal gas in the direction of SN1987A. In particular, two sets of authors^{1,2} have reported an absorption feature centred at 6379.70 Å corresponding to a velocity for an [Fe X]-line rest wavelength of 245 km s^{-1} , which is consistent with that of the LMC. The equivalent width of the line is reported as $\sim 2.5 \times 10^{-3}$ Å, implying a total Fe column density (including all ion stages) of $N_{\text{Fe}} \geq 10^{17} \text{ cm}^{-2}$. The corresponding HIM column density is

$$N_{\text{HIM}} \geq 3.2 \times 10^{21} \xi^{-1} \text{ cm}^{-2} \quad (4)$$

This HIM column density exceeds the neutral-hydrogen column density of $N_{\text{HI}} = 2.75 \times 10^{21} \text{ cm}^{-2}$ (Waite 1988 as quoted in ref. 2). Comparison with the X-ray-derived HIM column density derived from equation 3 leads to the relation

$$n_e^{-1} \delta \geq 2 \times 10^4 \text{ cm}^3 \quad (5)$$

Also, we have

$$N_{\text{HIM}} \sim n_e h \quad (6)$$

where h is the scale height of the X-ray emitting gas. These relations allow us to constrain the origin of the HIM responsible

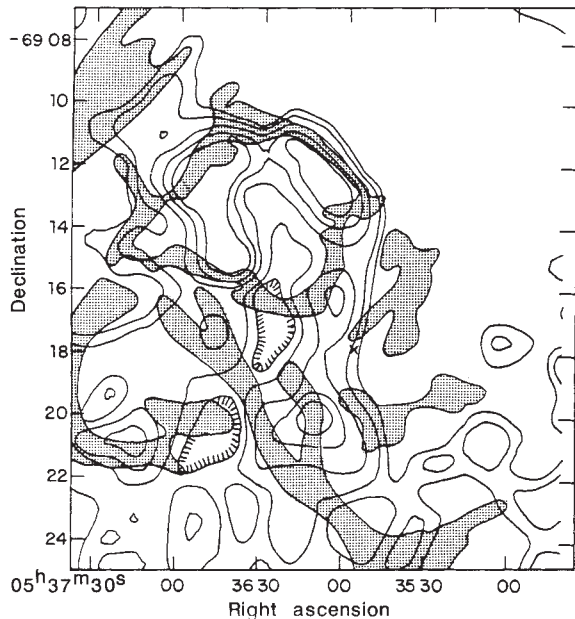


FIG. 1 A background-subtracted iso-intensity X-ray contour map for the SN1987A field with $H\alpha$ filaments indicated by shading. The X-ray data refer to the Einstein energy band (0.15–3.5 keV) and have been processed with a maximum-entropy algorithm. Contour levels are $(1, 1.2, 1.6, 2, 2.4) \times 10^{-4}$ counts $\text{arcmin}^{-2} \text{ s}^{-1}$. The cross in the centre marks the location of the supernova.

for the observed X-rays and essentially rule out the possibility that 6379-Å line represents Fe X absorption.

As stated above there is no indication that the ISM along the line of sight to SN1987A differs significantly from that in the surrounding region. Models in which the absorbing iron exists in a region small compared to the resolution of the IPC, models to which the above equations therefore do not apply, can be excluded by other considerations. In particular, we consider the case in which the observed iron was created in SN1987A itself, or in a previous, nearby SN. The optical light curve of the supernova has been used to infer that $0.07 M_{\odot}$ of iron was created and ejected in the explosion⁹, and current theoretical calculations show that the mass of iron ejected in type II events varies little ($\sim 15\%$) with the mass of the progenitor¹⁰. With this quantity of iron, the column density N_{Fe} required to explain the optical absorption line demands that the dimension of the ejecta be < 1 pc. The observed velocity width of the absorption line, however, is $\leq 60 \text{ km s}^{-1}$, whereas γ -ray¹¹ and infrared¹² spectra of the SN1987A mantle reveal a velocity dispersion in the iron layer of $\sim 3,000 \text{ km s}^{-1}$. If the iron were from the remnant of some previous supernova along the line of sight, our requirement for a path length of < 1 pc would suggest a very young (< 500 yr) object for which there is no other evidence and in which velocities would greatly exceed the observed line width. A circumstellar origin for the line is also ruled out² by several orders of magnitude. Thus, the possibility of producing the large iron column density in a single, highly localized source is excluded.

Alternatively, the observed coronal gas could be the integrated product of a period of locally enhanced star-formation activity, such as a giant bubble created by the nearby OB associations⁴. The combined action of stellar winds from high-mass stars and multiple supernovae create a hot, low-density cavity with a diameter D given by $D \approx 130 (N_{\text{SN}200} t_6^3 n_0^{-1})^{1/5} \text{ pc}$ where $N_{\text{SN}200}$ is the number of stars capable of producing supernovae normalized to 200 (ref. 13, 14), t_6 is the age in units of 10^6 yr and n_0 is the original mean density of the ISM. If we include an enrichment resulting from the production of $0.1 M_{\odot}$ of Fe in each of 200 SN over 5×10^7 yr, the expected column density of iron can be expressed as

$$N_{\text{Fe}} \sim 6 \times 10^{54} N_{\text{SN}200} t_6 D^{-2} \\ \sim 5 \times 10^{13} N_{\text{SN}200}^{3/5} n_0^{-2/5} t_6^{-1/5} \text{ cm}^{-2}$$

which is several orders of magnitude below the observed value

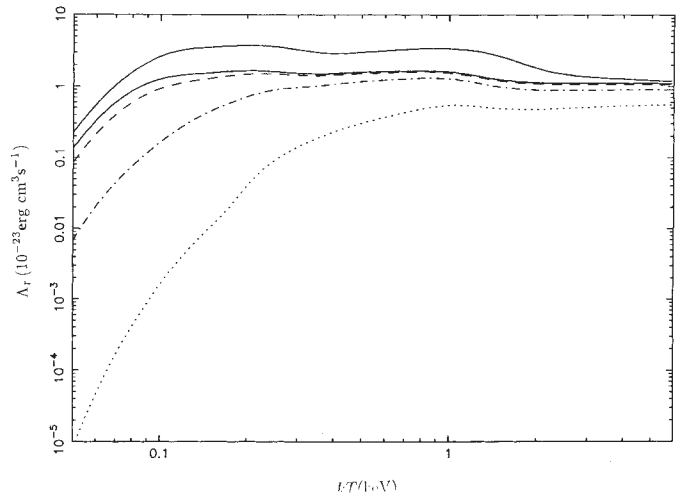


FIG. 2 Integrated X-ray emissivity in the Einstein energy band (0.15–3.5 keV) as a function of the HIM temperature. The top curve gives the function for normal solar abundances, whereas the lower three curves are for one-half solar abundance. To illustrate ISM absorption effects, we have included the observed emission through ISM column densities of 5×10^{19} , 5×10^{20} and $5 \times 10^{21} \text{ cm}^{-2}$ as the dashed, dash-dot and dotted curves respectively.

and is a decreasing function of the lifetime of the bubble.

Ultimately, such a bubble can evolve to a dimension comparable to the scale height of the dense ISM in the galactic plane whereupon it will merge into the halo of the LMC. We now examine the possibility that the [Fe X] line originates in such a hot LMC halo. The volume occupied by halo gas would greatly exceed the resolution of the IPC and we can use equations (2) through (5) to constrain severely the physical properties of the gas. In this case, the H I absorbing column density in the LMC cannot affect the observed X-ray emission; that is, $\delta \sim 25$ and we have from equation (5) $n_e \leq 10^{-3} \text{ cm}^{-3}$. Thus, from equation (6) we require $\xi h \geq 2,000 \text{ kpc}$ or forty times the distance to the LMC using cosmic abundances. For an assumed disk mass of $10^{10} M_\odot$ and a disk radius of 3 kpc, the 10^6-K HIM would have a scale height of $\sim 1 \text{ kpc}$ and ξ is constrained to be $\geq 10^3$.

As it is clear that the general ISM does not have this iron abundance, it is necessary to locate the absorbing material in a hot coronal layer that is $\geq 50\%$ iron. Such a layer would have a scale height of a few hundred parsecs and a cooling time of

$\sim 10^5 \text{ yr}$. The nucleosynthetic yield of type I supernovae is adequate to create such a layer in 10^8 yr , although it is difficult to see how the required temperature ($\sim 10^6 \text{ K}$) could be maintained consistent with an upper limit to the absorption line width of 60 km s^{-1} .

Finally, we consider the independent constraint on the HIM in the LMC imposed by the radio pulsar 0529-66. The dispersion measure for this object is $125 \text{ cm}^{-3} \text{ pc}$ (ref. 15) and, assuming it is in the LMC, this value sets an upper limit on the electron column density in any coronal gas between the Milky Way and the LMC. Subtracting the galactic contribution of $1.6\text{--}4.0 \times 10^{20} \text{ cm}^{-2}$ (ref. 15), we find $N_{\text{Fe}} \leq 10^{16} \xi \text{ cm}^{-2}$. This value, already more than an order of magnitude below the reported Fe X column density for solar abundances, is an extreme upper limit in that gas at much lower temperatures ($\sim 10^4 \text{ K}$) is likely to contribute substantially to the electron column density. Again, we would require an extremely high iron abundance in the coronal gas if this constraint is to be met. $\square$

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Short-time large-scale restructuring of the solar photosphere

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SOLAR granulation consists of small-scale brightness variations on the Sun's surface, caused by horizontal inhomogeneities in the temperature and density of the photosphere¹. Individual granules are typically 1,000 km across and have lifetimes of about 5 minutes, but the two-dimensional pattern has generally been considered stationary, apart from small deviations over the solar surface and variations during the solar cycle². Here I show that the nature of the granulation pattern changes on a timescale of 5-10 minutes (ref. 3). The number of granules increases coherently by a factor of 1.2-1.6 over an area of $(4\text{--}6) \times 10^8 \text{ km}^2$, accompanied by a change in the total granular area of a factor of 1.4 and a change in the mean area by a factor of 2, in antiphase with the number of granules. When the number of granules is increasing, formation of new granules by fragmentation exceeds their disappearance by merging by a factor of 3.6, whereas when the number of granules is decreasing merging occurs twice as fast as fragmentation. The mechanism for these changes is unclear.

I have studied the evolution of solar granulation in the quiet photosphere near the centre of the solar disk (heliocentric angle, $\theta = 21^\circ$) over a 15-min period. Nine direct photographs of the solar photosphere were taken by the Soviet Stratospheric Solar Observatory during its flight of 30 July 1970 (09:10:50-09:25:50 UT)⁴. Observations were carried out automatically at an altitude of 20 km. The telescope aperture was 500 mm, and an excellent spatial resolution of 0.25 arcsec could be achieved.

Photometrically calibrated photographs of superior quality were selected for processing. Isophotal maps were constructed using a digital microphotometer. Brightness is defined in terms

of deviations ΔI relative to the mean brightness level of the photosphere.

I have defined a granule to be a single bright region for which ΔI is everywhere greater than an arbitrary threshold value of -4% . The closed isophote nearest to this threshold bounds the area of the granule. For $\Delta I > 6\%$, a single granule can include several maxima (ref. 5). This precise definition of the granule makes it possible to distinguish single granules as structural elements on the isophotal maps and photographs and to estimate granular areas with high accuracy and uniformity.

The whole area A under investigation ($6.4 \times 10^8 \text{ km}^2$) was divided into nine equal areas ($l = 1\text{--}9$). The time t_k was measured from the first frame.

Figure 1 shows the temporal dependence of the number of granules $N_l(t_k)$ for the individual areas (bold lines) and for the average area $N_A(t_k)$ (fine line).

$$N_A(t_k) = \frac{1}{9} \sum_{l=1}^9 N_l(t_k)$$

Figure 1 and the photographs in Fig. 2 suggest that for all areas there is a similar systematic variation in the number of granules with time, but this visual impression requires statistical justification. The correlation coefficient of the variations in the number of granules over these nine areas is > 0.74 , that is, significantly different from zero (confidence level, $1 - \alpha \geq 0.95$) for six areas (the exceptions are $l = 6, 7, 8$). The variation of the granule number for the area B formed by averaging these six areas,

$$N_B(t_k) = \frac{1}{6} \sum_l N_l(t_k), \quad l = 1\text{--}5, 9$$

is shown in Fig. 3a.

I have assumed the variation of the number of granules with time to have the following form, incorporating a stochastic component:

$$N_l(t_k) = n_0 + \kappa_l n(t_k) + \nu_{kl}$$

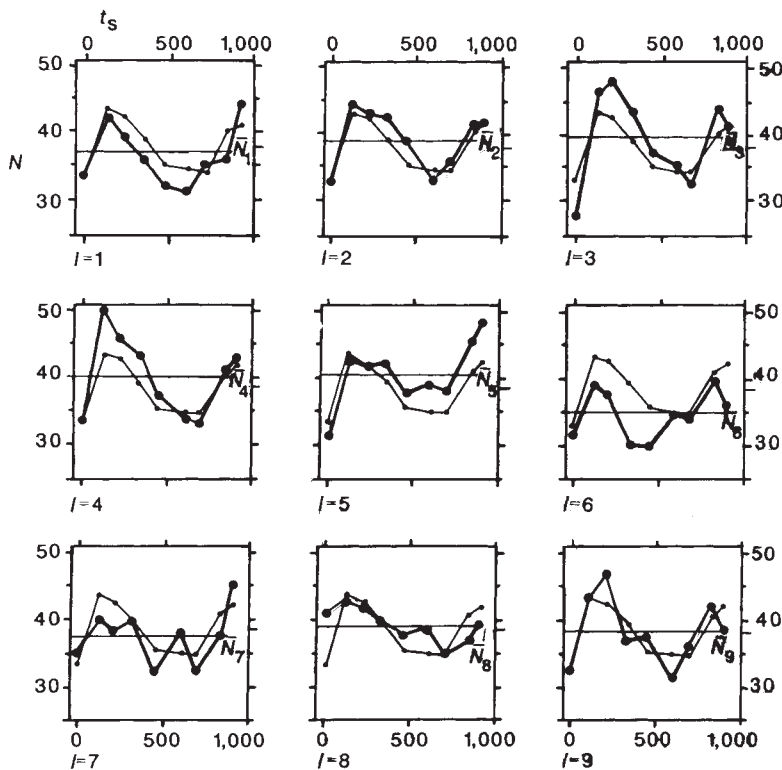


FIG. 1 The variation of the number of granules with time for nine equal sub-areas ($8,333 \times 8,500$ km) of the whole area A ($25,000 \times 25,500$ km) $N_l(t_k)$ (heavy line), and that for the average area $N_A(t_k)$ (thin line). Here $k=1-9$ is the frame number and t_k is the time of the frame at the start of the series. The mean number of granules for each area l , $\bar{N}_l$, is $\frac{1}{9} \sum_{k=1}^9 N_l(t_k)$, and for average area A, $\bar{N}_A$, is $\frac{1}{9} \sum_{l=1}^9 \bar{N}_l$.

Here n_0 is a constant, $\kappa_l n(t_k)$ is the time variation for the area l and κ_l is the amplitude relative to the mean for area B ($\sum_{k=1}^9 n(t_k) = 0$ and $\frac{1}{9} \sum_{l=1}^9 \kappa_l = 1$). ν_{kl} is a statistical component (noise). I assume that $l=1-5$, $9 \sigma\{\nu_{kl}\} = \sigma$ is constant and that the ν_{kl} are uncorrelated for its root mean square different areas and frames as well as being uncorrelated with the systematic variations $n(t_k)$. I estimate the variables

$$\hat{n}_0 = \bar{N}_B = \frac{1}{9} \sum_{k=1}^9 N_B(t_k) = 39.2$$

$$\hat{n}(t_k) = N_B(t_k) - \bar{N}_B \quad (\text{Fig. 3a})$$

$$\hat{\sigma} = \hat{\sigma}\{N_l(t_k) - N_B(t_k)\}, \quad \hat{\sigma} = 2.5, \quad k=1-9, \quad l=1-5, 9$$

The dashed lines in Fig. 3a mark the range $N_B \pm 3\hat{\sigma}/\sqrt{6}$. Hence it follows that: (1) The systematic coherent variations of the number of granules in the areas $l=1-5, 9$ (the total area is equal to 4.3×10^8 km²) are real. The confidence level of its deviation from random variations is $1 - \alpha \geq 0.99$. The number of granules varies by a factor of 1.2-1.5 in 2-5 min. (2) The noise, $\hat{\sigma} = 2.5$, is significantly less than that expected in the case of the poissonian distribution $\hat{\sigma}_p = \sqrt{\hat{n}_0} = 6.2$. (3) The systematic variations of the number of granules in the areas $l=6-8$ are less pronounced due to the small amplitude of κ_l and to the 2.5-fold increase in the noise for $l=8$.

The time variation of the number of granules $N(t_k)$ (Fig. 3b), the total granule area $S(t_k)$ (Fig. 3c), the mean granule area $s(t_k) = S(t_k)/N(t_k)$ (Fig. 3d) and the deviation of peak brightness from the mean (Fig. 3e) have been estimated for a family of granules in area C, corresponding to $l=1, 2$ and occupying an area of 0.7×10^8 km². Levels $N = \frac{1}{9} \sum_{k=1}^9 N(t_k) = 39$ (solid line) and $N \pm 3\hat{\sigma}$ (dashed line) are indicated in Fig. 3b.

The qualitative changes that alter a granule as an individual structural element are: the birth of a new granule; its fading away; the fragmentation into two or several granules; and the merging of the two or several granules. During the time intervals between such events a granule remains qualitatively unchanged but its area, brightness and other quantitative characteristics may change. The mean time intervals between the different kinds of events as well as variations of the total and mean granular areas associated with them have been also determined. The sections of the N , S and s curves (Fig. 3b, 3c, 3d) where the values are increasing, decreasing and constant were analysed separately.

The main results for the area C may be summarized as follows: (1) The number of granules in the area C increases by 1.5 times during a time interval of 300 s from its minimum ($t_0 = 600$ s) to its maximum ($t_0 = 900$ s). This is significantly greater than the scatter that is due to noise ($\alpha < 10^{-3}$). At the same time, the

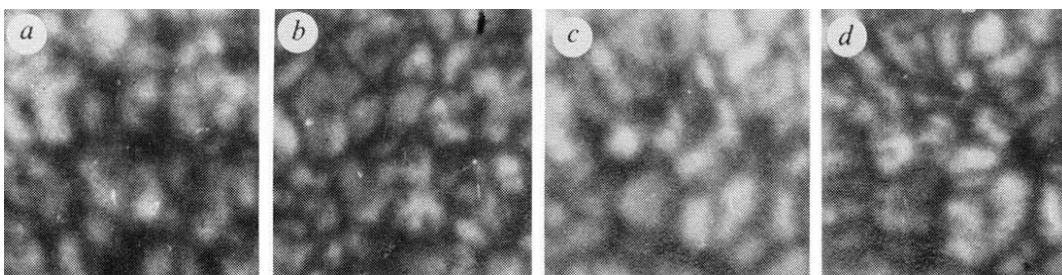


FIG. 2 The variation in granulation structure for area $l=3$. a, $t_k=0$ s, b, $t_k=210$ s, c, $t_k=690$ s, d, $t_k=840$ s.

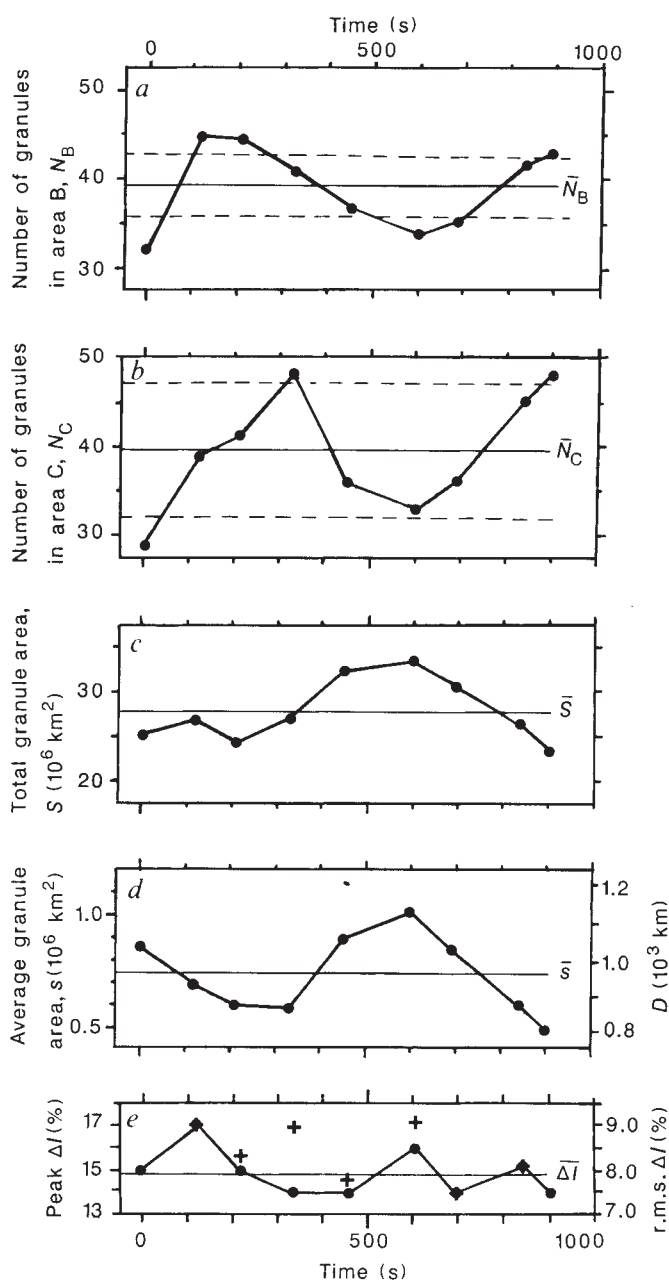


FIG. 3 The variation of the solar granulation characteristics. *a*, The time dependence of the number of granules for area B, averaged over the six sub-areas $l=1-5, 9$. Also shown are the mean number of granules, $\bar{N}_B = \frac{1}{9} \sum_{k=1}^9 N_{B_l}(t_k)$ (thin line), and the limits of the range, $\bar{N}_B \pm 3\sigma/\sqrt{6}$ (dashed lines) which indicates the spread about $\bar{N}_B$ that is due to the statistical noise (dashed lines). *b*, The time dependence of the number of granules for the area C, $N_C(t_k)$. Also shown are the mean number of granules in area C, $\bar{N}_C = \frac{1}{9} \sum_{k=1}^9 N_C(t_k)$, and the limits of the range $N \pm 3\sigma$, which indicates the spread about $\bar{N}_C$ that is due to the statistical noise (dashed lines). *c*, The total area occupied by the granules of area C, $S(t_k)$. The mean total area $\bar{S} = \frac{1}{9} \sum_{k=1}^9 S(t_k)$ is also shown. *d*, The time dependence of the mean granule area for area C, $s(t_k) = S(t_k)/N(t_k)$. The time-averaged mean granule area, $\bar{s} = \frac{1}{9} \sum_{k=1}^9 s(t_k)$, is also shown. *e*, *D* is the corresponding diameter for a circular granule. *e*, The mean peak-brightness deviation of the granules of the area C, relative to the mean brightness of the photosphere ΔI (line). The root-mean-square brightness contrast for the area A r.m.s. ΔI (crosses) is also shown. The mean brightness of the photosphere is assumed as 100%.

total granule area decreases similarly and consequently the mean granular area decreases by a factor of two (Fig. 3*b-d*). Correlation between the number of granules and their mean area is very high ($r = -0.89$). If the observed changes are periodic the possible period should be ~ 10 min. (2) The fragmentation and merging of a granule is five times as frequent as its birth or fading away. The mean lifetime of a granule as an individual structure is 5–6 min. (3) The balance of granule fragmentation and merging is not maintained when the number of granules is changing. When the number of granules is increasing, fragmentations are three times as frequent as mergings but when N_C is decreasing, fragmentations are twice as rare as mergings. (4) Granule merging is accompanied mainly by growth of their total area, and fragmentation is accompanied by a decrease. Thus there is a decrease of the total granulation area with an increase in their number and vice versa. When the granules are not changing qualitatively they exhibit a tendency to decrease their area with time, not to increase it as has been supposed⁶. (5) Variations in the mean granule area are primarily due to changes in the number of granules rather than to changes in the total area.

These variations cannot be explained by differences in the image qualities of the various photographs. The conventional measure of image sharpness when studying granulation is the root-mean-square (r.m.s.) brightness contrast on the photograph, r.m.s. ΔI . For our images this value is large (r.m.s. $\Delta I = 7.8-9.1\%$ for a wavelength of 465 nm)¹, it varies little between photographs and it does not correlate with either the number of granules or with their mean area. The same type of behaviour is shown by the peak ΔI which is also rather sensitive to a decrease in the spatial resolution and to measurement errors (Fig. 3*e*). This implies a high quality and uniformity of the photographs employed in the study. Tests were conducted which revealed that the presence of some blurring of the images does not appreciably change the number of granules. Two prints were made from one of the sharpest photographs ($t_k = 210$ s), one of which was deliberately defocused to blur it to a greater degree than was likely to occur for any of the actual photographs. Counting the number of granules by the adopted technique gave similar results for both the sharp and the defocused frames. I therefore conclude that the observed collective behaviour of this substantial ensemble of granules is genuine. The restructuring occurs over an area that is 1,000 times larger than that of a single granule^{1,5} and has been followed over a time of 300 s which is similar to the lifetime of a granule. The changes result from the combination of complex nonstationary processes which are, in a number of cases, asymmetric with respect to time inversion. These results imply a connection between the fine structure of the solar atmosphere and the large-scale, possibly global, processes on the Sun. This relationship has not been taken into account in gas-dynamics simulations of granulation⁷⁻⁹. The temporal behaviour should be taken into consideration when estimating changes of the granulation characteristics during the solar activity cycles.

My conclusions have been drawn from a single set of data only. How representative they are of the photosphere as a whole and the scale and mechanism underlying the relevant processes remain to be clarified. □

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Influence of nitrogen fertilization on methane uptake in temperate forest soils

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METHANE, a long-lived gas (8–10 years residence time), is important in the chemistry of the atmosphere and the Earth's radiation balance^{1–3}. The tropospheric abundance of CH₄ has been increasing by ~1.1% yr⁻¹ over the past decade^{4,5}. The cause of this increase may be due to either increases in global sources or decreases in global sinks^{1,6,7}. Although considerable research has focused on measuring CH₄ emissions from major biological sources^{7,8}, much less is known about the magnitude of, and factors controlling, biological sinks of CH₄. The largest biological sinks for methane are microorganisms in aerobic soils⁷. Here we report a study of CH₄ uptake by aerobic temperate-forest soils. We measured CH₄ consumption rates (up to 3.17 mg CH₄-C m⁻² day⁻¹) that were higher than reported previously. Globally, soils of temperate and boreal forests may consume up to 9.3 Tg CH₄-C yr⁻¹. We also found that the CH₄ uptake rates of these soils were decreased significantly by elevated soil moisture (14%) and nitrogen additions (33%), implying that nitrogen fertilization may reduce this CH₄ sink.

In the spring of 1988, nine (25 m²) plots were established in each of two forest stands at the Harvard Forest in central Massachusetts: a 62-year-old red pine (*Pinus resinosa* Ait.) plantation and an ~80-year-old mixed black oak/red maple (*Quercus velutina* Lam./ *Acer rubrum* L.) stand. Both stands were established on Gloucester-series soils following pasture abandonment in the early 1900s. Forest-floor soils in both stands are acidic, with pH values of 3.2 and 3.3 in the pine and hardwood stands, respectively. In each stand, three plots served as controls, three received a total of 37 kg N ha⁻¹ yr⁻¹ (low-N plots), and three received a total of 120 kg N ha⁻¹ yr⁻¹ (high-N plots). Fertilizer, added as NH₄NO₃, was applied in six equal additions from April to September. The total N applied to the low-N plots was four times the amount these forests normally receive in wet deposition^{9,10}, but was less than or equal to the amount received by forests in polluted areas of western and central Europe^{11–13}.

Measurements of CH₄ fluxes between soils and the atmosphere were made using 0.065-m², two-piece PVC-plastic chambers to cover the soil surface. The lower half (anchor) of the chamber was permanently implanted 1 cm into the forest floor in April 1988. One anchor was established in each plot. Flux measurements were conducted simultaneously at each of the nine plots in both stands, allowing us to examine both spatial and temporal variability among the various treatments. Air and soil (0–2.5 cm and 2.5–5.0 cm) temperatures were measured during each incubation. Soil moisture was analysed gravimetrically and available soil nitrogen was determined¹⁴.

Methane fluxes were measured six times (every four hours) over a 24-hour period by sealing the chamber top to the anchor (total chamber volume ~5.4 l) for a 30-min incubation. Gas samples (20 ml) were withdrawn from the headspace at the onset of incubation and at 10-min intervals thereafter. Chamber tops were removed at the conclusion of each incubation. A Poropak-Q column and a flame-ionization-detection gas chromatograph were used to separate and analyse methane from the headspace gas samples. Fluxes were calculated by a linear fit of concentration data as a function of the incubation time.

Methane uptake rates were measured in both stands for all treatments from May through October 1988 and the mean uptake rates observed over the sampling period were 0.13 mg CH₄-C m⁻² h⁻¹ in the hardwood control plots and 0.11 mg CH₄-C m⁻² h⁻¹ in the pine control plots. The monthly uptake rates shown in Fig. 1 are the means of rates measured from the biweekly samplings.

Observations of CH₄ consumption in temperate and boreal forests are few, but our study suggests that aerobic soils in these systems are more important than considered previously. Our uptake rates are about ten times greater than measurements in another New England temperate-forest ecosystem, where rates of 0.18 mg CH₄-C m⁻² d⁻¹ were reported¹⁵. Consumption rates comparable to ours have been found in the Great Dismal Swamp forest only during drought conditions, when the soils became aerobic. Some evidence from the literature indicates that dry tropical-forest soils are also CH₄ sinks^{15,17,18} with rates of ~0.18–0.37 mg CH₄-C m⁻² d⁻¹.

The amount of atmospheric CH₄ consumed by soils of tropical, temperate and boreal forests was estimated using rates from this study and ref. 15 for temperate and boreal forests and rates from refs 17 and 18 for tropical forests. The uptake rates were assigned functional periods of 365, 200 and 120 days in tropical, temperate and boreal systems, respectively, corresponding to the approximate number of frost-free days in these systems. Current estimates (1980) of the areas of tropical-, temperate- and boreal-forest ecozones were developed from ref. 19 and used with the above uptake rates to calculate the magnitude of the individual ecozone sinks.

A new finding based on this analysis is that temperate and boreal forests may play a much larger role than tropical forests

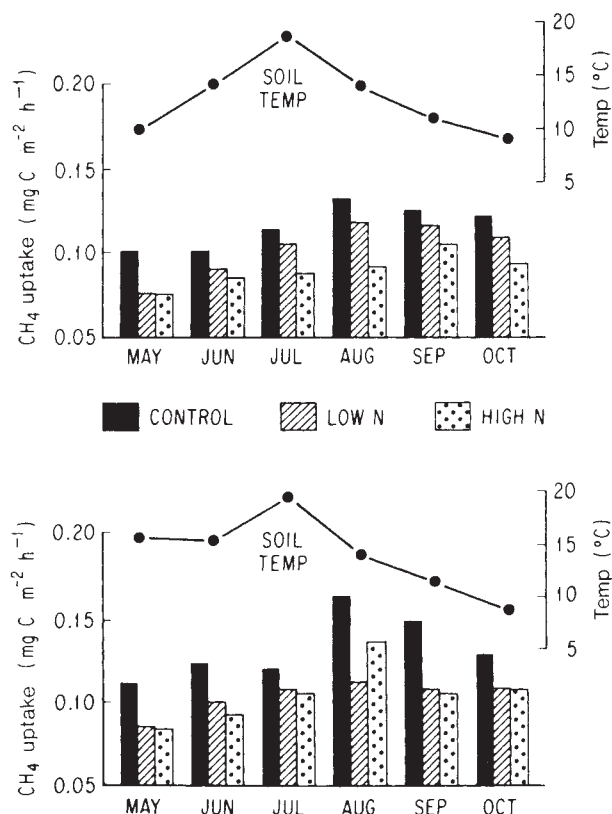


FIG. 1 Mean monthly CH₄ uptake and soil temperature (0–2.5 cm) for pine (top) and hardwood (bottom) stands at Harvard Forest. Low-nitrogen-fertilized plots received 37 kg N ha⁻¹ yr⁻¹ and high-nitrogen-fertilized plots received 120 kg N ha⁻¹ yr⁻¹.

in the consumption of atmospheric CH_4 (Table 1). We estimate that temperate and boreal forests annually consume up to 9.3 Tg $\text{CH}_4\text{-C}$ ($\text{Tg} = 10^{12} \text{ g}$), an amount 3.7 times larger than the 2.5 Tg $\text{CH}_4\text{-C}$ consumed by tropical forests. Our estimate for tropical forests is less than the 4 Tg $\text{CH}_4\text{-C yr}^{-1}$ reported in refs 17 and 18 because we used a smaller ecozone area ($18.5 \times 10^{12} \text{ m}^2$) in the calculation. There is considerable uncertainty in estimating the magnitude of the CH_4 sink for these systems, however, because of the limited number of measurements in tropical forests, the lower reported rates for temperate forests¹⁵ and the difficulty in assessing the effects of soil temperature, moisture and nitrogen status on the consumption rates in all the systems.

Methane uptake showed no correlation with the soil temperature over the sampling period May to October (Fig. 1). Linear regression analysis of consumption rates with soil temperature showed no correlations: pine $r^2 = -0.005$; hardwood $r^2 = 0.022$.

We observed that both the moisture conditions and the nitrogen status of the sites affected the magnitude of the sink. The effect of soil moisture conditions on CH_4 uptake is demonstrated by comparing the 11 July rates to the 25 July rates (Fig. 2). Lower uptake rates (paired t -tests $\alpha < 0.001$) were measured in all plots during the 25 July sampling when soil moisture was increased significantly (analysis of variance (ANOVA) $P < 0.01$). This is consistent with other observations^{15,16}. The mechanism responsible for effects of moisture on CH_4 uptake has been discussed¹⁵. Briefly, the organisms that oxidize methane include methanotrophs^{20,21} and also nitrifiers²². Both methane oxidizers and methane-producing bacteria (methanogens) are thought to be widespread in soils. In fact, they often exist in the same areas. Methanogens are obligate anaerobes, occupying deeper, wetter soil horizons, whereas the methane oxidizers (obligate aerobes) occupy surface, aerobic horizons. Changes in soil moisture result in changes in the net balance of methane production and consumption with wetter conditions shifting the balance towards production and/or reduced gas exchange with the atmosphere.

An important and previously unreported finding of this study is that increased soil nitrogen content resulted in lower CH_4 uptake rates (Fig. 1). After only four months of fertilization, methane consumption in the fertilized plots of both stands was reduced by up to 15% in the hardwood stand and 24% in the pine stand. Analysis of the consumption rates after six months of fertilization indicates that the difference becomes more pronounced—the consumption rates were reduced by ~33% in the high-N plots relative to the controls. We found that, over the six-month sampling period, uptake rates in the fertilized plots of both stands were reduced significantly compared to the control plots (ANOVA $P < 0.005$). The long-term trajectory of this relationship, however, is not well known.

TABLE 1 Estimates of CH_4 uptake by tropical, temperate and boreal forests

Ecozones	Area (10^{12} m^2)	CH_4 uptake range ($\text{g C} \times 10^{-4} \text{ m}^{-2} \text{ d}^{-1}$)	$\text{Tg}^* \text{ C yr}^{-1}$ (min. -max.)
Tropical forests			
Tropical moist	10.83	1.87–3.75	0.74–1.48
Tropical seasonal	7.66	1.87–3.75	0.52–1.05
		Total tropical forests	1.26–2.53
Temperate & boreal forests			
Temperate evergreen	4.88	1.87–27.5	0.18–2.68
Temperate deciduous	4.41	1.87–31.7	0.16–2.80
Boreal	11.60	1.87–27.5	0.26–3.83
		Total temperate and boreal forests	0.60–9.31

* $\text{Tg} = 10^{12} \text{ g}$.

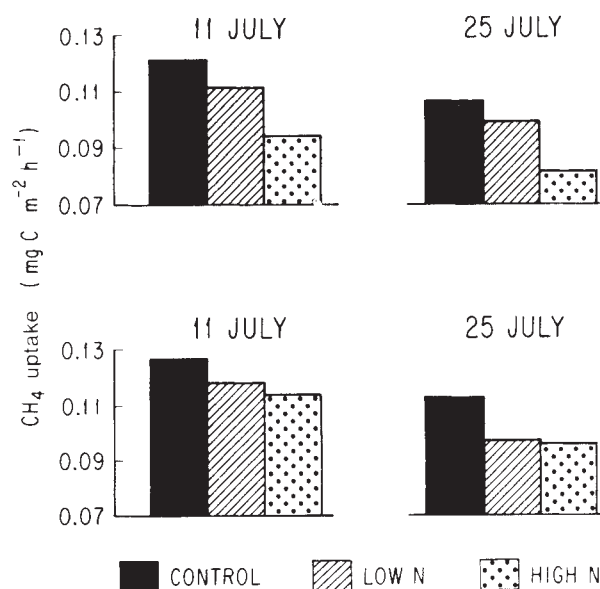


FIG. 2 Methane uptake by control and nitrogen-fertilized soils of pine and hardwood forests (top and bottom, respectively) at Harvard Forest, Massachusetts. Measurements made before (11 July 1988) and immediately after (25 July 1988) a 6-cm rain event. Soil temperatures (0–2.5 cm) ranged from 20.1 to 17.5 °C in the pine stand and 20.6 to 18.2 °C in the hardwood stand on 11 July and 25 July, respectively.

The mechanism responsible for the effect of nitrogen on CH_4 uptake is complex because it involves both methanotrophs and nitrifiers. Laboratory studies indicate that oxidation of CH_4 by a variety of methanotrophs is competitively inhibited by nitrogen, especially ammonium^{20,23–25}.

Nitrifying bacteria, including the dominant soil ammonium-oxidizing bacterium *Nitrosomonas europaea*, have the ability to oxidize CH_4 , even at low atmospheric concentrations^{26,27}. Further, laboratory cultures using *N. europaea* showed reduced CH_4 oxidation activity²⁶ at ammonium concentrations (15 p.p.m.) equivalent to or higher than those present in soils at our sites. The results of these laboratory studies are consistent with our field observations and suggest that CH_4 oxidation may be suppressed in systems that receive high inputs of nitrogen, such as agroecosystems and acid-rain-affected temperate and boreal forests. The identification of a link between nitrogen inputs and CH_4 uptake rates is especially important in understanding the role that aerobic soils play in the global atmospheric methane cycle. The global-scale consequences of a reduction in the CH_4 sink with nitrogen fertilization are uncertain because of the lack of data on the generality of the methane–nitrogen interaction. Further measurements in agroecosystems, pastures and in systems receiving high nitrogen inputs from deposition are necessary to clarify the nitrogen–methane interaction before extrapolation to a global basis. □

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Evidence for sulphate-controlled phosphorus release from sediments of aquatic systems

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SEDIMENTS of aquatic systems play a critical part in controlling phosphorus loading to the water column¹. Because P loading is an important determinant of productivity in aquatic systems, there has been keen interest in variables that influence P release from sediments. In disagreement with present theories^{1,2} our data from 23 different aquatic systems indicate that sulphate concentration of waters is an extremely important variable controlling P release from sediments. The increased P release from sediments at higher sulphate concentrations may help to explain why primary production in freshwater systems (with relatively low sulphate concentrations) tends to be P limited³, whereas in many saline systems (with high sulphate concentrations) production is often P sufficient⁴. Further, our results indicate that anthropogenically induced changes from atmospheric S inputs could, over time, alter the P cycle of aquatic systems.

To examine the controls of P release from sediments, without the possible artefacts of sediment core studies, we used an *in situ* geochemical approach^{5–8} (Fig. 1, legend). Briefly, sediment P immobilization is inferred when measured P release is less than that expected from decomposition reactions. Particles reaching bottom sediments show P:C ratios that vary between 3 and 10 mmol per mol C (refs 9 and 10); when the ratio of dissolved P to dissolved inorganic carbon (DIC) release is equal to this value, the decomposition reactions alone may be adequate to explain P release from sediments. Release ratios below 3 mmol P per mol C indicate that sediment uptake is significant, whereas ratios above this value indicate that there is P release from sediments that was taken up previously by sorption, mineral formation or bacterial uptake^{7,8}. The advantage of this approach is that it allows us to distinguish between two very different cases: (1) low sediment P release that is due to low availability of degradable organic materials (hence low metabolism); and (2) low sediment P release that is due to the fact that sediments have the capacity to take up P and prevent its release to overlying waters. That is, low absolute P release is not necessarily due to the capacity of sediments to immobilize P (case 1). The ratio of P release to inorganic carbon release (relative P release; RPR) is a far better indicator of the capacity of sediments to immobilize P.

Previous reports suggest that oxygen, through its control on the iron cycle, is the critical variable controlling P immobiliz-

ation by sediments of aquatic systems^{1,2}. The oxygen-control model states that under oxic conditions a micro-layer rich in iron oxide traps P, preventing its release into overlying waters, whereas under anoxic conditions these iron oxides are reduced chemically and previously bound P is released into solution². If this model represents the dominant processes occurring in sediments, RPR would be consistently low (<3–10 mmol P per mol C) under oxic conditions and high (>3–10 mmol P per mol C) under anoxic conditions^{7,8}. Our results do not show any such consistent pattern. In the 23 systems examined, we found a large variation in RPR under both oxic (0.1–10 mmol P per mol C) and anoxic conditions (0.1–35 mmol P per mol C). The difference in RPR between oxic and anoxic conditions is, therefore, small in comparison with the large variation in RPR among systems.

Clearly, some variable(s) other than oxygen must have a major influence on RPR. Our preliminary data and previous reports indicate that such variable(s) are related strongly to the ionic strength of waters^{5–8,11}. We therefore tested several variables thought to influence sediment P release that could be correlated with ionic strength (Table 1)^{12–18}. Of these variables, sulphate concentration of surface waters was the only variable with predictive significance ($P < 0.01$) under both oxic and anoxic conditions (Table 1).

Our data show that RPR and sulphate concentration of waters are related and that a relationship exists independent of whether bottom waters are oxic or anoxic (Fig. 1). Under oxic conditions RPR increased monotonically from 0.1 to 10 mmol P per mol C over the entire range of sulphate values tested (10–30,000 μM). Anoxic RPR showed a similar pattern to oxic RPR but had a steeper initial slope and reached maximum values at sulphate concentrations of only 100–200 μM (Fig. 1). Considering the entire data set for both oxic and anoxic conditions, three patterns emerge: Group I, brackish and saline systems, with high sulphate concentrations ($\sim 3,000$ – $30,000 \mu\text{M}$), have high RPR values (3–10 mmol P per mol C) under both oxic and anoxic conditions. In these systems, even under oxic conditions, immobilization of sediment P is generally not large; Group II, fresh water systems with low sulphate concentrations (below about 60 μM) generally have low RPR values (less than 1 mmol P per mol C) under both oxic and anoxic conditions. In these systems, P immobilization in sediment evidently continues to be important even after bottom waters become anoxic; Group III, fresh water systems of intermediate sulphate concentration (~ 100 – $300 \mu\text{M}$) generally have fairly low RPR under oxic conditions but tend to have very high RPR when bottom waters become anoxic. These are the only systems that correspond to the classic pattern² of P immobilization under oxic conditions and P release under anoxic conditions.

TABLE 1 Spearman rank-correlation coefficients between relative P release (RPR) and several variables that might be responsible for controlling P release

	Conductivity	SO_4^{2-}	pH	P:C	Sediment Fe	Chlorophyll
Oxic	0.75*	0.85*	0.57*	0.39	0.12	0.19
Anoxic	0.44	0.69*	0.41	0.34	0.20	0.38

* 'Oxic' refers to RPR when water above the sediments is oxygenated; 'Anoxic' refers to RPR when overlying waters are anoxic.

* Significance at the 99% level. P:C is the phosphorus-to-carbon ratio of suspended particles (siston) in surface waters. This ratio may be a reasonably good indicator of P:C ratios for freshly sedimented particles⁹. Sediment Fe is the iron content of upper 5–10 cm of the sediments. Chlorophyll represents mean surface-water value of chlorophyll *a* during the summer season. Twenty-three aquatic systems are included in this analysis; for data sources see Fig. 1. The range of values represented from these systems are: conductivity, 18–40,000 $\mu\text{S cm}^{-1}$; sulphate, 11–27,000 μM ; pH, 5.5–8.5 (H^+ , 0.0032–3.2 μM); sediment Fe, 0.9–4.5% dry weight; chlorophyll 1.5–60 $\mu\text{g l}^{-1}$.

The observed relationship between sulphate and RPR suggests that sulphate may have a role in the control of P uptake by sediments. Although as yet we do not know the mechanism(s), the causality of such a relationship is supported by some experimental evidence indicating that sulphate additions can inhibit P binding by sediments^{13,18}. Also, there are several plausible mechanisms whereby sulphate concentrations could influence the ability of sediments to take up P: mechanism (1), sulphate could compete with phosphate for anion sorption sites. This effect may be important in inhibiting P sorption by sediments in brackish and saline systems with extremely high sulphate concentrations (Group I systems; 3,000–30,000 μM)¹². Sorption competition, however, is unlikely to explain the differences in RPR observed among fresh water lakes (Group II and III systems; 10–300 μM sulphate; Fig. 1). The difference in RPR

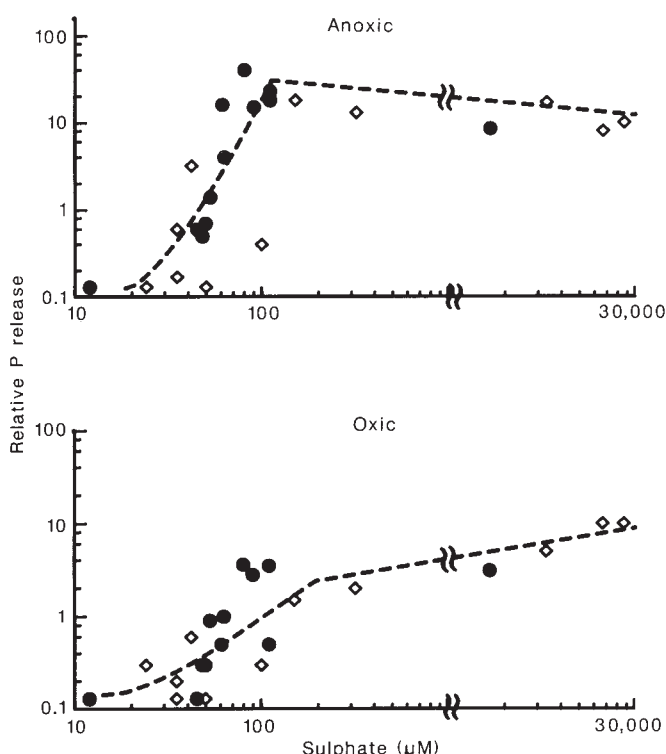


FIG. 1 Relative P release (RPR) plotted against sulphate concentration of surface waters for 23 different aquatic systems. Release under both oxic (bottom) and anoxic (top) conditions are shown. Data for 12 of the 23 systems shown are from our data (closed circles). Data for the remaining 11 systems (open diamonds) are from D. W. Schindler (personal communication), K. Porter (personal communication) and from refs 5–8 and 23–27. METHODS. During summer stratification, water was collected from below the thermocline of lakes on 2–6 occasions using a peristaltic pump. Water for analysis of total dissolved P was filtered in line through a Whatman GFF filter and later analysed²⁸. Samples for dissolved inorganic carbon (DIC) and CH_4 were collected into airtight (BOD) bottles and measured by gas chromatography²⁹. Oxygen was measured using a YSI oxygen meter. Sampling in lakes occurred under both oxic (early season) and anoxic (late season) conditions. Oxic RPR is the P:DIC accumulation ratio from apparent oxygen utilization of zero to oxygen concentrations of 5% saturation (~ 300 – 400 μM of DIC accumulation). Anoxic RPR is the same ratio from 5% oxygen saturation to a DIC accumulation of up to 800 μM (but >400 μM) due to anaerobic decomposition processes. Methane was not included in the calculation of RPR. This exclusion can lead to underestimations of decomposition under anoxic conditions and, thus, overestimations in RPR. For the 11 systems for which we had methane data, however, this error was small in comparison with the range in RPR. Because methanogenesis is of greater importance in low sulphate systems³⁰, however, this error would only enhance the observed pattern of increases in RPR at higher sulphate concentrations.

under anoxic conditions is particularly pronounced in these systems and two more mechanisms, both depending on sulphate reduction, may be operative; mechanism (2), sulphides (from sulphate reduction) could bind iron in anoxic sediments^{13,19}. Formation of iron sulphides would both prevent the re-supply of iron oxides to surface sediments¹⁹, and the formation of ferrous phosphate compounds within anoxic sediments¹³. This mechanism implies that RPR should be related directly to sulphate reduction and sediment iron content; mechanism (3) sulphate reduction in anoxic sediments or bottom waters can generate alkalinity and elevate pH; high pH values would inhibit P sorption onto iron oxides in sediments¹². This mechanism implies that RPR should be related to hypolimnetic pH (an indirect effect of many factors including sulphate reduction) and sediment iron content.

In a recent study using enclosures, mechanism (3) was shown to be important for a soft-water lake in Ontario¹⁸. This same study indicated that mechanism (2) was not important. Our results from a wide range of lakes indicate, however, that this result may not be applicable generally across systems. Using multiple regression analysis we were able to explain 84% of the variation in RPR under anoxic conditions using sulphate depletion in bottom waters and sediment iron content as the independent variables (Table 2). For the same data set, we were able to explain only 20% of the variation in RPR under anoxic conditions using hypolimnetic pH and sediment iron content as independent variables. These results, taken together, suggest that sulphate reduction may be acting to influence RPR and that the dominant mechanism is not related to pH in bottom waters¹⁸. More work on sediment and pore water chemistry is needed to elucidate fully the mechanisms (and several may be operative) whereby sulphate may be affecting P cycling in aquatic systems.

Whatever the mechanism, the correlation between sulphate and P release from sediments may help to explain a well-known difference between fresh- and saltwater systems. In freshwater lakes P is, in general, the major nutrient limiting phytoplankton growth³; in coastal marine systems, on the other hand, phytoplankton production is usually P sufficient and N is limiting to phytoplankton growth^{4,20}. This difference is attributed generally to changes in N cycling between fresh and salt waters^{20,21}. Our data indicate that P is probably most available in systems with

TABLE 2 Multiple regression models of relative P release (RPR) into anoxic hypolimnia of twelve fresh water lakes

Independent variables	Number of variables	r^2 (adjusted)	p value
Sulphate depletion, sediment Fe	2	0.81	0.002
Sulphate, sediment Fe	2	0.67	0.003
Hypolimnetic pH, sediment Fe	2	0.02	0.36
Table 1 variables including sulphate	6	0.77	0.023
Table 1 variables excluding sulphate	5	0.00	0.74

In all regressions RPR was log transformed to stabilize the variance. We were able to obtain significant relationships only if either sulphate or sulphate depletion were included in the suite of independent variables. Combinations excluding sulphate but including hypolimnetic pH or surface-water pH did not result in significant relationships. RPR (mmol P per mol C) was best predicted from sulphate depletion in bottom waters (μM) and sediment Fe (% dry weight) according to the equation: $\ln(\text{RPR}) = 0.11(\text{sulphate depletion}) - 0.97(\text{sediment iron}) - 0.006$. Of the variation in RPR explained by this equation, 75% was due to sulphate depletion and 25% was due to sediment iron. Data were included for all fresh water systems for which, in addition to the variables listed in Table 1, we had sulphate depletion and hypolimnetic pH. Data are from this study (9 lakes), from D. W. Schindler (personal communication), from ref. 23 (2 lakes) and from refs 24 and 25 (1 lake). Sulphate depletion is the maximum observed sulphate depletion above sediments that occurs during summer stratification. Similarly, hypolimnetic pH is the pH value above the sediment water interface at the end of summer stratification. All other variables are explained in Table 1.

very high sulphate concentrations (Fig. 1). Thus, differences in P cycling between freshwaters and salt waters may also influence the switch in nutrient limitation.

A further implication of our findings is a possible effect of anthropogenic S pollution on P cycling in lakes. Our data indicate that aquatic systems with low sulphate concentrations have low RPR under either oxic or anoxic conditions; systems with only slightly elevated sulphate concentrations have significantly elevated RPR, particularly under anoxic conditions (Fig. 1). Work on the relationship between sulphate loading and

P release has yet to prove the mechanism behind this relationship. If sediment P release were controlled largely by sulphur, our view of the lakes that are being affected by atmospheric S pollution could be altered. It is believed generally that lakes with well-buffered watersheds are insensitive to the effects of atmospheric S pollution. However, because changing atmospheric S inputs can alter the sulfate concentration in surface waters²² independent of acid neutralization in the watershed, the P cycle of even so-called 'insensitive' lakes may be affected. □

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Routing of meltwater from the Laurentide Ice Sheet during the Younger Dryas cold episode

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ROOTH¹ proposed that the Younger Dryas cold episode, which chilled the North Atlantic region from 11,000 to 10,000 yr BP, was initiated by a diversion of meltwater from the Mississippi drainage to the St Lawrence drainage system. The link between these events is postulated to be a turnoff, during the Younger Dryas cold episode, of the North Atlantic's conveyor-belt circulation system which currently supplies an enormous amount of heat to the atmosphere over the North Atlantic region². This turnoff is attributed to a reduction in surface-water salinity, and hence also in density, of the waters in the region where North Atlantic Deep Water (NADW) now forms. Here we present oxygen isotope and accelerator radiocarbon measurements on planktonic foraminifera from Orca Basin core EN32-PC4 which reveal a significant reduction in meltwater flow through the Mississippi River to the Gulf of Mexico from about 11,200 to 10,000 radiocarbon years ago. This finding is consistent with the record for Lake Agassiz which indicates that the meltwater from the southwestern margin of the Laurentide Ice Sheet was diverted to the northern Atlantic Ocean through the St Lawrence valley during the interval from ~11,000 to 10,000 years before present (yr BP).

Evidence for these diversions of meltwater comes from changes in the level of proglacial Lake Agassiz, which acted as a 'clearinghouse' for runoff from a $2 \times 10^6 \text{ km}^2$ area along the southwestern side of the Laurentide Ice Sheet³. Before 11,000 yr BP, the meltwater reaching Lake Agassiz overflowed to the Gulf of Mexico through the Mississippi River drainage system (Fig. 1a). By ~11,000 yr BP the Laurentide Ice Sheet had retreated far enough to open a series of channels leading to the Lake Superior basin, creating a diversion of the Agassiz

TABLE 1 Key radiocarbon dates of wood

Moorhead low-water phase of Lake Agassiz*

10,960 ± 300 (W-723)	10,340 ± 170 (I-5213)
10,820 ± 190 (TAM-1)	10,200 ± 80 (GSC-1909)
10,680 ± 190 (GSC-677)	10,080 ± 280 (W-900)
10,550 ± 200 (Y-411)	10,050 ± 300 (W-1005)

Marquette glacial advance into Superior basin†

10,250 ± 250 (W-1541)	10,100 ± 100 (WIS-409)
10,230 ± 300 (W-3896)	9,850 ± 300 (W-3866)
10,230 ± 500 (W-1414)	9,780 ± 250 (W-3904)
10,220 ± 215 (DAL-338)	9,730 ± 140 (I-5082)
10,200 ± 500 (M-359)	9,545 ± 225 (DAL-340)
10,100 ± 250 (WIS-409)	

Emerson high-water phase of Lake Agassiz‡

10,000 ± 280 (GSC-1428)	9,890 ± 300 (I-4853)
10,000 ± 150 (GSC-870)	9,880 ± 225 (GX-3696)
9,990 ± 160 (GSC-391)	9,820 ± 300 (W-1361)
9,940 ± 160 (I-3880)	9,810 ± 300 (W-1360)
9,930 ± 280 (W-388)	9,700 ± 140 (GAC-797)
9,900 ± 400 (W-993)	

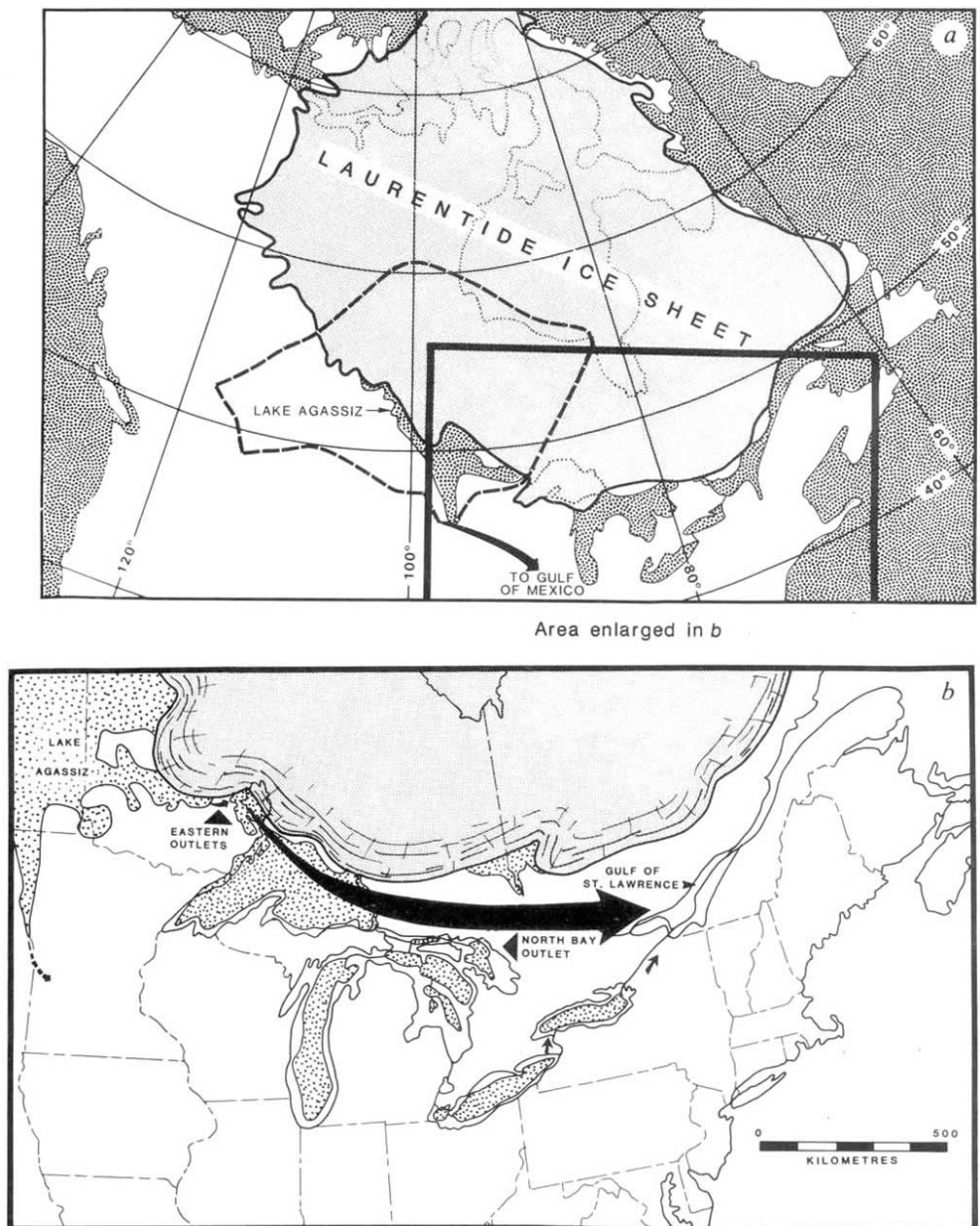
These dates (yr BP) were used to establish the chronology for the routing of meltwater from Lake Agassiz drainage basin during the Younger Dryas cold episode. The sample numbers are given in brackets.

* Low-water phase during which meltwater flowed east to the northern Atlantic Ocean.

† Glacial advance that dammed eastern overflow from Lake Agassiz.

‡ High-water phase during which meltwater flowed south to Gulf of Mexico.

FIG. 1 *a*, Map showing Laurentide Ice Sheet and routing of overflow from the Lake Agassiz basin (dashed outline) to the Gulf of Mexico just before the Younger Dryas^{22,23}; *b*, Routing of overflow from Lake Agassiz through the Great Lakes to the St Lawrence and northern Atlantic during the Younger Dryas²⁴.



water eastward through the Great Lakes and the St Lawrence valley to the northern Atlantic (Fig. 1*b*). Thus, during the Younger Dryas, meltwater and precipitation from the Lake Agassiz basin, the discharge of which is estimated⁴ to have been $\sim 30,000 \text{ m}^3 \text{ s}^{-1}$, was diverted to the northern Atlantic Ocean from its previous route to the Gulf of Mexico. This diversion caused the level of Lake Agassiz to drop by $\geq 40 \text{ m}$, creating the Moorhead low-water phase⁵. Radiocarbon dates of material from the portions of the floor of Lake Agassiz exposed during the Moorhead low-water phase range from 10,960 to 10,050 radiocarbon years (Table 1).

Drainage from Lake Agassiz was routed back to the Mississippi River and Gulf of Mexico beginning $\sim 10,000$ years ago, when ice of the Marquette glacial advance once again dammed the eastern overflow channels that had linked Lake Agassiz with the St Lawrence during the time corresponding to the Younger Dryas. Lake Agassiz again rose at this time to its Mississippi outlet initiating the Emerson high-water phase. Radiocarbon dates related to both the ice advance in the Superior basin and the Emerson high-water phase are shown in Table 1. Eleven radiocarbon ages for the ice re-advance range from 10,250 to

9,545 yr and average 10,020 yr. Eleven radiocarbon ages for the Emerson high-water phase of Lake Agassiz range from 10,000 to 9,700 yr.

An appropriate independent test for these reconstructions of drainage history is to date meltwater influx episodes to the Gulf of Mexico, recorded in the sedimentary record as negative values in the $\delta^{18}\text{O}$ of planktonic foraminifera^{12,13}. Because of bioturbation effects however, necessary resolution is not attainable for sediments that accumulate at rates of $8 \text{ cm per } 10^3 \text{ yr}$. The Orca Basin, located on the continental rise off Louisiana, not only offers high sedimentation rates ($50 \text{ cm per } 10^3 \text{ yr}$) but also anoxic conditions and hence a lack of sediment mixing¹⁴⁻¹⁷. There is^{14,15} a major negative $\delta^{18}\text{O}$ spike in Orca Basin core EN32-PC6. Accelerator radiocarbon dating of this core conducted on planktonic foraminifera¹⁸ revealed what seemed to be a sedimentation hiatus covering the time interval of the Younger Dryas. A more recent radiocarbon age of $10,910 \pm 160 \text{ yr}$ (or $10,500 \text{ yr}$ after correction for the $\sim 400\text{-yr}$ age for surface water ΣCO_2) for a new sample of mixed planktonic foraminifera from 436–437 cm depth in EN32-PC6, however, supports the contention that accumulation was continuous¹⁷.

Because of our original suspicion that a hiatus existed in EN32-PC6 we turned to a companion core, EN32-PC4, and determined the oxygen isotopic composition of both the white and pink variety of *Globigerinoides ruber* (Fig. 2). The isotope record was radiocarbon dated using the accelerator ^{14}C method (Table 2 and Fig. 2). Both ^{18}O records show the meltwater anomaly¹²⁻¹⁵. The white *G. ruber* record shows a sharp increase ($\sim 3\%$) in $\delta^{18}\text{O}$ at $\sim 11,000$ yr BP which is consistent with the cessation of meltwater flow expected from a diversion of the Lake Agassiz water. This increase is followed by a sharp decrease ($\sim 2\%$) in $\delta^{18}\text{O}$ at $\sim 10,000$ yr BP which is consistent with the rejuvenation of meltwater flow when the Lake Agassiz discharge was returned to the Mississippi River system.

Had we stopped with the analysis of the white variety of *G. ruber* we would have concluded that the evidence from the Gulf of Mexico is in full agreement with the expectation based on the Lake Agassiz record. The analyses of pink forms of *G. ruber*, however, complicates the situation. Although they show the major $\delta^{18}\text{O}$ increase corresponding to the eastward diversion 11,000 yr BP, they show no decrease at 10,000 yr BP. Rather, the $\delta^{18}\text{O}$ value remains nearly constant from 11,000 yr BP to present. Another puzzling contrast between these two records is that the $\delta^{18}\text{O}$ value for the white form of *G. ruber* is 1‰ higher than that for the pink form during glacial and Younger Dryas times, whereas the two forms yield similar values before and after Younger Dryas time. These differences are not easily explained. Net-tow¹⁹ plankton sampling and isotope²⁰ studies suggest that the pink form of *G. ruber* lives only during summer months, whereas the white form lives throughout the year. As the largest meltwater flux to the Gulf of Mexico would certainly have been during the summer months, this would be the least probable time for the Orca Basin to have been free of meltwater effects.

The important question here is whether the difference between the records invalidates our claim for verification of the cessation event. For example, a strong winter cooling ($\sim 6^\circ\text{C}$) during the Younger Dryas would explain the trough in the $\delta^{18}\text{O}$ record in white forms of *G. ruber*. In this case no increase in meltwater

TABLE 2 Radiocarbon dates of mixed planktonic shells

Sample No.	Depth (cm)	^{14}C age (yr)	Corrected ^{14}C age* (yr)
F120	94-96	9,100 $\pm$ 190	8,700
F81	144-146	10,490 $\pm$ 90	10,100
F82	150-152	10,700 $\pm$ 130	10,300
F83	164-166	11,030 $\pm$ 90	10,600
F84	192-196	11,510 $\pm$ 100	11,100
F85	214-216	12,450 $\pm$ 90	12,000
F121	288-290	12,900 $\pm$ 150	12,500

These are the ages, determined by accelerator mass spectrometry at ETH Zurich, of mixed planktonic shells from Orca Basin core EN32-PC4 ($26^\circ 56.1' \text{ N}$, $91^\circ 21.7' \text{ W}$; 2,260 m).

* Corrected for air-sea $\Delta^{14}\text{C}$ difference.

flow down the Mississippi 10,000 yr BP would be required. Indeed, faunal data have been interpreted as indicating a cooling of the Gulf of Mexico during the Younger Dryas cold episode¹⁷. However, no support for a large cooling has been found in the pollen record for the southeastern United States (see ref. 21 for a summary).

Although it is unclear how much of the change in the $\delta^{18}\text{O}$ observed in the white form of *G. ruber* is due to reintroduction of meltwater and how much to a temperature increase, the chronology of the oxygen isotope record in the Gulf of Mexico seems to be in harmony with that for the record of Lake Agassiz. Although further tests of this hypothesis are required, the fact that the Younger Dryas interval is bracketed by dramatic oxygen isotope changes in the Gulf of Mexico is strong support for the proposal that a major influx in meltwater to the northern Atlantic ocean was responsible for the Younger Dryas cold episode.

Independent oxygen-isotope evidence from the Bay of Biscay (France) for benthic foraminifera²⁵ and a new coral-based chronology for the sea level during the time interval 15,000-9,000 yr BP²⁶ suggest that the Younger Dryas interval was one of reduced meltwater flow relative to the warm intervals before and after this event. The problem is that as meltwater from other sectors of the Laurentide Ice Sheet and from the Scandinavian Ice Sheet entered the Northern Atlantic at all times, it might be claimed that the increase in fresh-water input to the northern Atlantic at the onset of the Younger Dryas cold episode caused by the diversion of meltwater from the Mississippi to the St Lawrence was largely offset by a reduction in meltwater input from these other sectors. A further issue regarding the Rooth hypothesis is that the re-division of meltwater from the Mississippi to the St Lawrence after 10,000 yr BP failed to produce a cold event comparable to the Younger Dryas. Although we do not have satisfactory answers to these questions, we feel that the cadmium- and carbon-isotope data for benthic foraminifera²⁷ clearly show that the Atlantic conveyor-belt system did shut down during the Younger Dryas time. Taken together with meltwater-routine chronology we feel that the case for a diversion trigger remains strong. $\square$

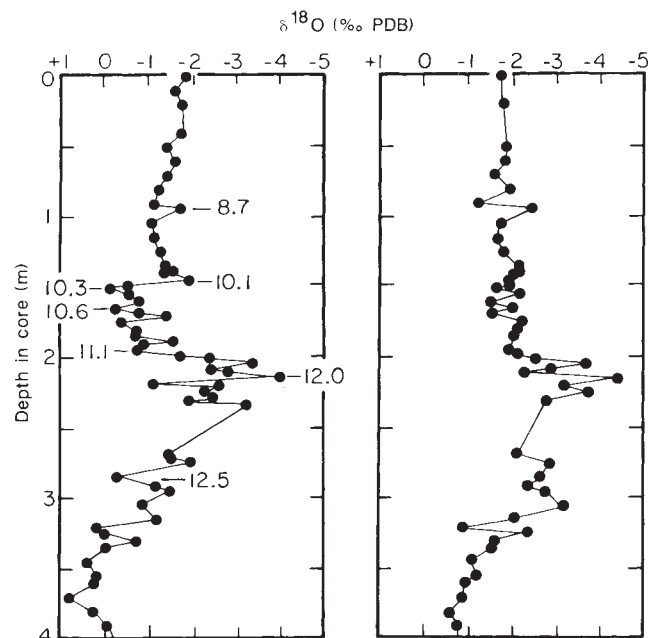


FIG. 2 Oxygen isotope changes in two varieties of *Globigerinoides ruber* (white and pink, left and right, respectively) from EN32-PC4 during latest Quaternary are plotted against depth. Ages shown are accelerator radiocarbon ages. Both varieties show a major 'meltwater spike' from ~ 320 -200 cm. *G. ruber* (white variety) shows increased $\delta^{18}\text{O}$ 1‰ relative to PDB standard values at the beginning of the Younger Dryas cold episode, consistently high values during the episode ($\sim 11,000$ -10,000 yr BP), and a sudden decrease at 10,000 yr BP termed the 'cessation event'.

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Stroboscopic NMR microscopy of the carotid artery

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THE non-invasive measurement of vascular dynamics and elasticity is critical in understanding haemodynamic conditions of cardiovascular diseases such as hypertension and atherosclerosis¹. Although there are numerous invasive and *in vitro* techniques² for such measurements, until now non-invasive methods have been limited³. We have now obtained stroboscopic NMR images^{4,5} of the carotid arteries of 80-g rats. The change in the cross-sectional area of arteries of diameter ~600–800 μm was correlated with the change in absolute blood pressure. These are the first microimages^{6–9} of a dynamic system and enable the direct visualization of compliance, the non-invasive measurement of Young's modulus, the direct determination of the local effects of vasoconstrictors and vasodilators and the mapping of the entire cardiac cycle.

Figure 1 (left panel) shows a large-field-of-view (FOV) spin-echo NMR image of the left side of the neck of an 80-g rat, whereas the images shown in the middle and right panels correspond to systole and diastole, respectively. The carotid artery at systole appears dark (corresponding to the absence of signal) because the blood that was excited during slice selection had flowed out of the slice by the time the echo was obtained. Fresh

unexcited blood had taken its place, thereby affording great contrast. The image at diastole is an extreme example of a complete loss of contrast that occurs when the blood flow is so slow that it is difficult to distinguish between the blood in the artery and the water in the surrounding tissue. This effect was observed only rarely (more representative images at diastole are shown in Fig. 3), and the echo time (T_E) could be adjusted to change the intensity of the blood signal in the artery.

Data such as these shown in Fig. 1 were analysed by drawing expanded plots, the contours of which correspond to the grey levels in the image. Representative plots are shown in Fig. 2. Each of the areas of these plots was measured independently by three individuals, using a planimeter. The averages of the three normalized measurements for two rats are shown in Fig. 3. The error bars correspond to the standard error of the three separate measurements. The error is small near systole (~2%) and larger near diastole (~8%) because of the flow effects mentioned above.

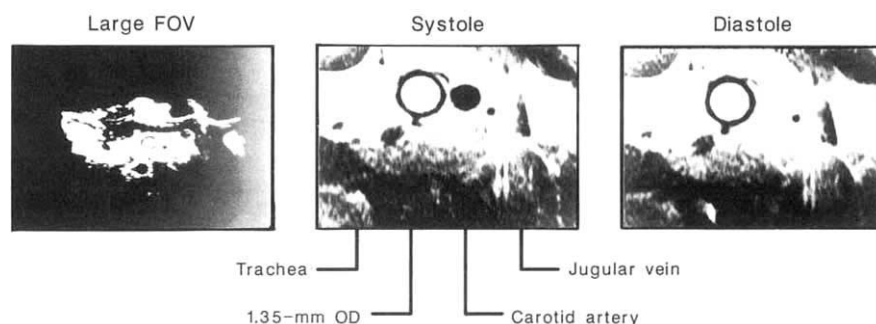
The cross-sectional areas of the carotid artery at the extremes of systole and diastole can be correlated with the absolute blood pressure to determine the distensibility, related to the compliance, of the carotid artery. Compliance is defined as the volume change per unit of pressure; our measurements, however, recorded a change in the cross-sectional area. We have assumed that the length of the carotid artery did not change appreciably during the heart cycle, an assumption that is valid in view of separate measurements of the transverse and longitudinal Young's modulus (see below). The distensibility is calculated by:

$$\frac{(A_{\text{systole}} - A_{\text{diastole}}) / A_{\text{diastole}}}{P_{\text{systole}} - P_{\text{diastole}}} \times 100\%$$

where P_{systole} and P_{diastole} are the absolute blood pressures at systole and diastole, respectively, and A_{systole} and A_{diastole} are

FIG. 1 Spin-echo NMR images of the left side of the neck of an 80-g rat. Left, Field of view (FOV) 33.9 × 33.9 mm; middle, FOV 8.5 × 8.5 mm; this image corresponds to systole. Right, FOV 8.5 × 8.5 mm; image corresponds to diastole.

METHODS. Female Sprague-Dawley rats (80 g) were anaesthetized with ethyl carbamate (urethane, Sigma) in 0.85% saline (w/v) by injecting 850 mg of the solution per kg (body weight) intraperitoneally, and then injecting 850 mg per kg subcutaneously behind the neck. The rats were intubated and a water-filled capillary (10 mm × 1.35 mm o.d.) was inserted near the carotid artery as a marker for vertical positioning. The incision was sutured and electrocardiograph leads were placed on the upper abdomen, away from the surface coil, with the resultant electrocardiogram producing a V_3 -like trace. The rat was placed head-down in a specially designed plastic rodent holder that was affixed to the probe. The temperature of the rats decreased to 31 °C and stabilized at that level. They breathed 60% O_2 and 40% N_2 . The NMR images were obtained using a Bruker AM 360 equipped with an NMR microscope accessory. The probe circuit was modified to accommodate a 1.4-cm-diameter single-turn surface coil. A 2-ms lobeless sinc pulse was used for the slice-selective 90° and 180° pulses. The water line-width was shimmed on the desired slice to ~100 Hz. The gradient strengths for the 33.9 × 33.9 × 0.8-mm FOV were 0.69, 0.74 and 1.8 gauss cm^{-1} for the



read-out (x), phase encode (y) and slice select (z) directions, respectively. For the 8.5 × 8.5 × 0.8-mm FOV the gradient strengths were 2.77, 1.48 and 1.8 gauss cm^{-1} , respectively. The images consisted of two scans at each of 64 phase-encode increments surrounding the echo maximum. The pixel size in the phase-encode direction was 132 μm , and 33 μm in the read direction before zero filling. The data matrix was zero filled to 512 × 512 before Fourier transformation; no signal enhancement or digital filtering was applied to the data. Slice thickness was ~800 μm . The repetition time (T_R) was 6 s and the echo time (T_E) was 20 ms.

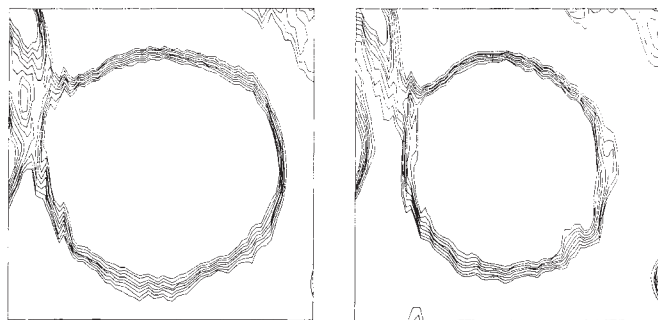


FIG. 2 Representative contour plots for cross-sectional area determinations.

the corresponding arterial cross-sectional areas. The average distensibility was determined as 1.1% per mm Hg (range 0.67–1.36%) from the NMR data for six rats.

These NMR data provided a unique opportunity to measure non-invasively the transverse Young's modulus of the carotid artery. The Young's modulus E is a measure of the elasticity of a material and increases as the material stiffens. It is defined by $E = \text{stress/strain}$, or

$$E = \frac{2\pi r_0}{s} k$$

(ref. 10) where r_0 is the artery radius at zero pressure and s is the corresponding thickness of the arterial wall. For a section of artery of length l , the constant k is related to the force tangential to the arterial wall F and to the pressure P exerted by the blood on the arterial wall by

$$F = Prl = kl[2\pi(r - r_0)]$$

where r is the arterial radius at pressure P . From this expression,

$$\Delta P = 2\pi k \Delta r / r$$

Integration gives

$$(P_{\text{systole}} - P_{\text{diastole}}) = 2\pi k [\ln(r_{\text{systole}}/r_{\text{diastole}})],$$

where r_{systole} and r_{diastole} are the arterial radii at systole and diastole, respectively. Thus, we could determine k from the available NMR data and once k was obtained, calculate r_0 by

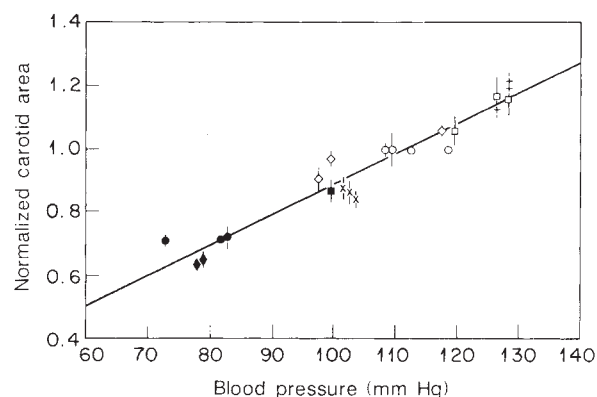


FIG. 4 Plot of the normalized cross-sectional area of the carotid artery versus blood pressure for five rats, with and without phenylephrine. The data before and after administration of the vasoconstrictor were fully reversible. ○, Control, systole; ●, control, diastole; +, drug dose 1, systole; ×, drug dose 1, diastole; □, drug dose 2, systole; ■, drug dose 2, diastole; ◇, post-drug systole; ◆, post-drug diastole.

METHODS. Blood pressure was measured using a catheter inserted into the right carotid artery. A 1.5-cm length of polyethylene tubing (0.61 mm OD, 0.28 mm ID, Clay Adams PE 10) was inserted into the artery; it was joined to a 2.9-m length of larger (0.965 mm OD, 0.58 mm ID, Clay Adams PE 50) polyethylene tubing, filled with a solution of normal saline with heparin (10 USP units ml⁻¹ saline), that led out of the magnet to the pressure transducer (Spectramed P23XL). The artery was periodically flushed with heparinized saline (0.1 ml). The blood pressure typically remained stable to ~5% systole and ~8% diastole during ~5 h total experimental time. The right jugular vein of the rat was catheterized with a 2.9-m length of polyethylene tubing (PE 10). This led to a calibrated syringe pump (Sage Instruments, model 355). For an 80-g rat, phenylephrine (Sigma; 1 mg ml⁻¹ in normal saline) was delivered at ~7.5 μl min⁻¹, with small variations needed to keep the blood pressure constant. For an 80-g rat, sodium nitroprusside (0.08 mg μl⁻¹; Sigma) in 5% dextrose (w/v) was delivered at 13 μl min⁻¹.

setting P_{diastole} to zero. The thickness of the artery at zero pressure must be known. We measured the average thickness of an excised artery from an 80-g rat using a light microscope as 0.10 ± 0.02 mm.

The average Young's modulus was calculated as 7×10^5 dyn cm⁻² for six rats (range 4.9×10^5 – 1.3×10^6 dyn cm⁻²).

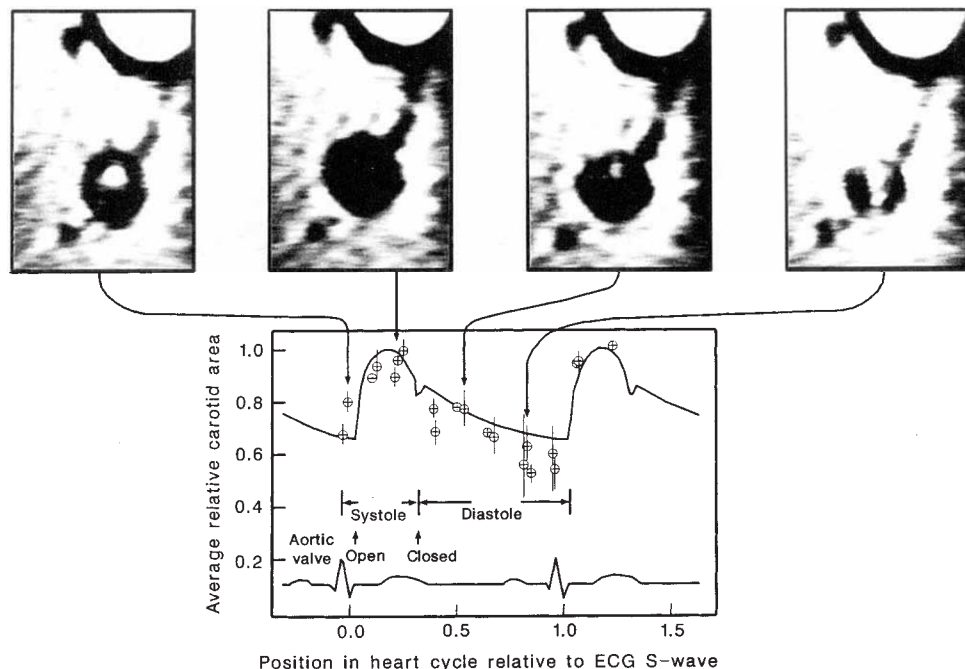


FIG. 3 Arterial cross-sectional area as a function of phase in the heart cycle. The line through the data corresponds to blood pressure in a human heart cycle and is intended only as a guide to the eye.

METHODS. Either the R- or the S wave of the electrocardiogram was used for gating. The spin-echo pulse sequence incorporated a time delay between the triggering and excitation. This delay was adjusted so that slice excitation corresponded to a known fraction of the heart cycle. The heart period (typically 150 ms) was recorded periodically and remained stable to within 3% during the 13-min experiment and ~10% during the 5 h typically required to acquire a series of data.

This can be compared with a value of $6.5 \times 10^3 \text{ dyn cm}^{-2}$ for the transverse modulus of an excised carotid from an 80-g rat, measured mechanically with a Rheovibron viscoelastometer. (The longitudinal Young's modulus was $3.1 \times 10^6 \text{ dyn cm}^{-2}$.) The agreement between the NMR measurement of Young's modulus and the classical Rheovibron measurement is remarkable, considering the errors associated with keeping the sample moist during the viscoelastic measurements, and the assumption that the arterial thickness is identical for all arteries.

The NMR method can also be used to provide non-invasive information about the local action of vasoactive drugs. The vasoconstrictor phenylephrine affects primarily the small arterioles. As the heart beats more slowly, this results in a compensatory increase in the cardiac output. The carotid artery, therefore, must expand to accommodate this increase in volume—such an expansion may be observed directly (data not

shown). Figure 4 shows the cross-sectional areas of the carotid arteries of five rats, normalized to the pre-drug systole area, plotted against the blood pressure, with and without phenylephrine. The linearity of the data shows that the drug raised the blood pressure without affecting the distensibility of the carotid artery. Similar experiments were performed with the vasodilator nitroprusside. Although the variation between rats was greater in these experiments, the data suggest that the dilation of the artery at diastole with the drug was greater than that without it.

The data shown here were obtained for the carotid artery of 80-g rats, whose arteries were $\sim 600\text{--}800 \mu\text{m}$ in diameter. We predict that it is possible to observe changes under high-contrast conditions when there are 4–5 pixels across the artery. This would set a lower limit of 2.5–3 mm for the diameter of human vessels that can be measured accurately, given the 0.5-mm resolution so far available on whole-body machines. □

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Cloned cytotoxic T cells recognize an epitope in the circumsporozoite protein and protect against malaria

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PROTECTIVE immunity against malaria is induced by vaccination of hosts with irradiation-attenuated sporozoites. This immunity is mediated in part by neutralizing antibodies that are directed mainly against the repeat domain of the circumsporozoite protein^{1–4}. Early experiments showed, however, that B-cell-depleted mice that are immunized with sporozoites can resist challenge, indicating that T-cell effector mechanisms may also have a role in protection⁵. This idea was supported by the recent observation that protective immunity also requires T-cells expressing the CD8 antigen (CD8⁺ T cells)^{6,7}, whose target is probably the developing liver-stage parasites^{8–10}. Moreover, an oral *Salmonella* vaccine that expresses the circumsporozoite protein is able to protect against murine malaria in the absence of antibodies¹¹. Here we report the identification of an epitope contained within amino acids 249–260 of the *Plasmodium berghei* circumsporozoite protein that is recognized by H-2Kd-restricted cytotoxic T cells^{12,13}. Passive transfer into mice of cytotoxic-T-cell clones that recognize this epitope conferred a high degree of protection against challenge. These results provide the first direct evidence that CD8⁺ T cells that are specific for a defined epitope can confer protection against a parasitic infection.

To identify epitopes in the *P. berghei* circumsporozoite (CS) protein recognized by cytotoxic T lymphocytes (CTLs), spleen cells from sporozoite-immunized BALB/c mice (haplotype H-

2d) were stimulated *in vitro* in the presence of a pool of 12 synthetic peptides¹⁴ (series I, Fig. 1) representing parts of the CS sequence. After 5–7 days of *in vitro* stimulation, the lytic activity of the spleen cells was assayed by incubation with P815 target cells (haplotype H-2d) in the presence of the individual CS peptides.

In initial experiments, the spleen cells lysed the P815 target cells only in the presence of the peptide corresponding to amino acids 249–260 (amino-acid sequence (single-letter code) NDDSYIPSAEKI). Control immune spleen cells cultured

TABLE 1 Cytotoxic activity of spleen cells from sporozoite-immunized mice

Origin of effector cells	<i>In vitro</i> expanded with peptide	E:T†	% Specific lysis of:			
			P815 cells*		EL-4 cells*	
			Peptide present	Peptide absent	Peptide present	Peptide absent
Immune spleen‡	Amino acids 249–260	30	39	10	1	1
		15	26	5	2	2
		7	17	2	1	0
Immune spleen	None	100	13	14	ND	ND
		30	7	5	ND	ND
		10	4	0	ND	ND
		3	2	1	ND	ND
Naive spleen	Amino acids 249–260	100	5	9	ND	ND
		30	1	4	ND	ND
		10	0	–1	ND	ND
		3	–1	–3	ND	ND

* ⁵¹Cr-labelled P815 target cells (2×10^3 , in 50 μl medium), or EL-4 target cells (1×10^4 , in 50 μl medium), were added to wells of V-bottom microtiter plates with or without peptide of amino acids 249–260 (1 μM). Viable spleen cells were collected after *in vitro* expansion, and added to each well in different numbers, at E:T ratios of 3:1–100:1. After 4 h of incubation at 37 °C, the plates were centrifuged and 100 μl of supernatant removed to measure chromium release. The per cent specific lysis was calculated as:

$$\frac{(\text{experimental release (c.p.m.)} - \text{spontaneous release c.p.m.})}{(\text{total c.p.m.} - \text{spontaneous release c.p.m.})} \times 100\%$$

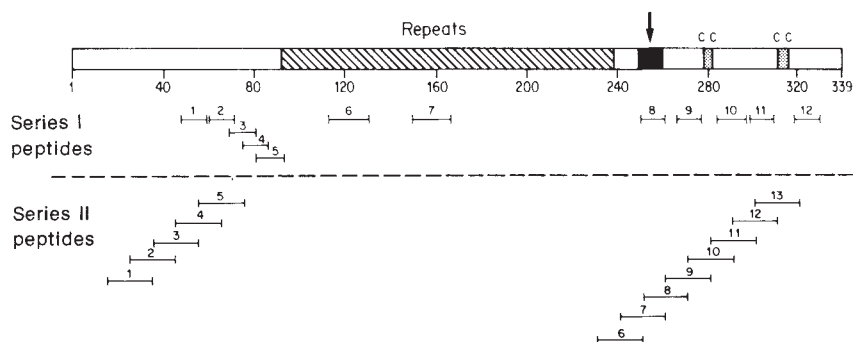
Each value represents the mean of duplicate wells.

† E:T, ratio of lymphocyte effector cells to target cells.

‡ Immune spleen cells were obtained from BALB/c mice that were immunized by multiple intravenous injections of irradiation-attenuated *P. berghei* sporozoites. Spleen cells were cultured *in vitro* for five days²⁵, in the presence or absence of 5 μM peptide of amino acids 249–260.

FIG. 1 Schematic representation of the *P. berghei* CS protein and related peptides. The sequences of the peptides used in these studies were taken from the deduced amino-acid sequence for the CS gene as reported^{12,13,28}.

Amino acids of peptides of series I are: 1, 47–58; 2, 59–70; 3, 68–80; 4, 74–85; 5, 80–92; 6, DPPPNNPNDPPPNNPND (single-letter code, dimeric repeat); 7, DPAPPNANDPAPPNAND (single-letter code, dimeric repeat); 8, 249–260; 9, 265–276; 10, 283–296; 11, 297–308; and 12, 317–328. Amino acids of peptides of series II are: 1, 20–39; 2, 30–49; 3, 40–59; 4, 50–69; 5, 60–79; 6, 240–259; 7, 250–269; 8, 260–279; 9, 270–289; 10, 280–299; 11, 290–309; 12, 300–319; and 13, 310–329. Amino acids of peptides of series II (except 1 and 3) have a C-terminal tyrosine that is not part of the protein sequence.



Peptides of series I and II were synthesized and purified as described^{17,29}. The arrow indicates the position of the cytotoxic epitope.

without peptide had no cytolytic activity. The recognition of this peptide was genetically restricted: only P815 target cells (haplotype H-2d), but not EL-4 target cells (haplotype H-2b), were specifically lysed. Naive spleen cells cultured for the same period of time with the 249–260 peptide had negligible lytic activity, indicating that the CTLs had not been primed *in vitro* by the peptide, but had been induced *in vivo* by immunization with sporozoites (Table 1).

A T-cell line was then obtained from the immune spleen cells by stimulation with the 249–260 peptide, and from this population 10 clones, designated series CS.B, were derived by limiting dilution. Four of these clones (cs.B-28, -31, -35 and -43) also killed the P815 target cells in the presence of the homologous peptide from the *P. yoelii* CS protein (amino acids 276–288), NEDSYVPSAEQI¹⁵, differing in the three underlined amino acids from the 249–260 *P. berghei* peptide.

An independent series of four clones (series CS.C) were derived from two other immunized BALB/c mice. In this case, the spleen cells were directly stimulated under limiting-dilution conditions with a pool of a new series of thirteen 20-mer peptides (series II, Fig. 1). The series-II peptides spanned most of the sequence of the CS protein, except for the repeats, and portions of the putative signal- and anchor sequences. The peptides of series II overlapped by 10 amino acids, and differed in size from the peptides of series I. The specificity of the CS.C clones was determined by incubating each clone with the P815 target cells and pools of three peptides. If the assay was positive, it was repeated using the individual peptides. All four CS.C clones recognized two overlapping peptides (amino acids 240–259 and 250–269), which included most of the amino-acid sequence 249–260, but not other peptides of series II. None of the CS.C clones recognized the *P. yoelii* peptide of amino acids 276–288.

These findings indicate that mice bearing the H-2d haplotype recognize predominantly a single cytotoxic epitope within the CS protein. Such a limitation in the number of CTL epitopes and their MHC-dependence has been found in other viral polypeptides^{16–21}.

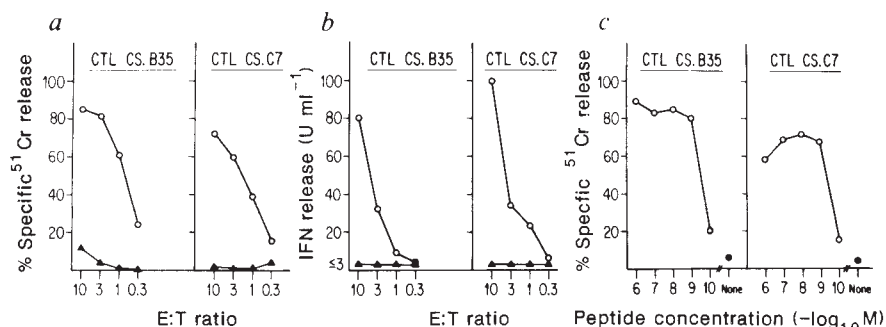
By cytofluorometry, all 14 CTL clones expressed the CD3 and CD8 antigens, but not the CD4 antigen (phenotype CD3⁺CD8⁺). To determine the class I MHC product involved in the recognition of the epitope of amino acids 249–260, we assayed the lytic activity of the clones on L-cell fibroblasts transfected with the *Kd*-, *Ld*-, or *Dd* genes of H-2 (ref. 22). In the presence of the 249–260 peptide, all of the clones were able to lyse only the H-2*Kd*-transfected cells. The lytic activity was inhibited specifically by monoclonal antibodies against the *Kd* antigen but not the *Ld* antigen (data not shown).

Determinations of the ratio of lymphocyte effector cells to target cells (E:T) that was required for lysis were carried out for most of the CTL clones. In a representative experiment (Fig. 2a), we observed a 50% specific lysis of target cells at E:T ratios as low as 1.0–0.3, in the presence of 1.0 μ M peptide. All clones released γ -interferon (γ IFN) into the culture medium during the 4-h assay (Fig. 2b). In other experiments, the E:T ratio was maintained constant at 3:1, and the concentration of the 249–260 peptide was varied. For many CTL clones, 50% specific lysis was obtained in the presence of as little as 10^{–9} M peptide (Fig. 2c).

To determine whether the CTL clones could protect against sporozoite-induced malaria infection, nine clones were expanded and adoptively transferred into groups of naive BALB/c mice. Eighteen hours after the T-cell clone transfer, the mice were challenged by intravenous injection of *P. berghei* sporozoites. In separate experiments, the clone CS.C7 con-

FIG. 2 Results of cytotoxic assays performed with P815 target cells and CTL clones in the presence of peptide 249–260. Specific lysis (a) and the production of γ IFN (b) at different E:T ratios, in the presence of 1.0 μ M peptide (○) or in the absence of peptide (▲, ●). (c), Specific lysis obtained with different peptide concentrations and a constant ratio of 3:1.

METHODS. A panel of 14 CS-specific CTL clones was obtained by using two different strategies. The series CS.B consisted of 10 CTL clones derived by limiting dilution of a short-term T-cell line. This T-cell line was obtained from spleen cells of sporozoite-immune mice, re-stimulated weekly with irradiated spleen cells that had been pulsed with peptide 249–260. Cells were grown in medium containing concanavalin A-induced T-cell growth factors²⁵. The cloning was performed by limiting dilution, using as stimulators irradiated P815 cells pulsed with peptide 249–260²⁶. Once the clones were established, they were maintained by weekly re-stimulation with the same stimulator cells in addition to peptide. The four clones of the series CS.C were obtained directly by limiting dilution of sporozoite-immune spleen cells, without previous *in vitro* expansion. The P815 stimulator cells were pulsed



with a mixture of thirteen 20mer peptides (series II, Fig. 1) for 1 h at 37 °C. These clones were expanded for the first three passages with the same mixture of peptides until their epitope specificity was determined. Cells were then maintained by weekly re-stimulation with P815 cells pulsed with peptide 249–260. The CTL assay was performed as described in the footnote to Table 1. To measure the production of γ IFN during the CTL assay, an aliquot of 30 μ l was removed from the supernatant after 4 h incubation. γ IFN content was measured using a previously described immunoassay³⁰.

sistently conferred a high degree of protection to the recipient mice (Table 2). Two other clones (CS.C1 and CS.C11) were also protective. Protection was obtained with challenge doses of 2×10^3 – 10^4 sporozoites when 2×10^7 cells of the CS.C7 clones were transferred. Protection was species-specific, as indicated by the failure of the CS.C7 clones to protect against challenge with 500 sporozoites of *P. yoelii*, a more infective rodent malaria parasite than *P. berghei*. This result is in agreement with the lack of *in vitro* recognition of the homologous *P. yoelii* peptide by the CS.C7 CTL clones (data not shown). Furthermore, the protective effect was also stage-specific; the CS.C7 clone conferred no protection against challenge with 2,000 red blood cells infected with *P. berghei*.

Partial protection, which needs to be confirmed by additional experiments, was observed with three other clones (CS.C3, CS.B43 and CS.B18). No protection was observed with clones

CS.B35, CS.B28 and CS.B83. Although differences in the protective ability of CTL clones have been reported in another system²³, the basis of this functional heterogeneity remains unclear. Variation in the fine specificity of the clones, or the loss of 'homing' receptors after long periods of *in vitro* culture may be responsible for this phenomenon²⁴. Furthermore, the method used in this study to evaluate the efficacy of the adoptive protection, that is the detection of patent infection, is very stringent and is based on an all-or-nothing effect. Even if a large number of the liver-stage parasites had been inhibited by the T-cell clones, a full-blown infection of the erythrocytes would result.

The most probable targets of the protective CTL clone are the intrahepatocytic stages of malaria parasites, the protection being mediated either through a direct recognition of the infected cells, or by the release of lymphokines. Gamma-IFN, a product of T-cells, has a potent inhibitory effect on the development of liver-stage parasites^{8,9}. But a recent study has shown that spleen cells from mice that have been immunized with irradiated sporozoites can eliminate *P. berghei* parasites from hepatocytes *in vitro*, and that this effect cannot be inhibited by a monoclonal antibody against γ IFN (ref. 10).

The processing of polypeptide antigens and their presentation to T-cells that recognize class-I-restricted epitopes seem to result from the intracytoplasmic degradation of the antigens (for a review see ref. 14). Sporozoites, however, are not localized in the cytoplasm, but are found in endocytic vesicles. Perhaps the CS protein leaks into the cytoplasm of the host cells (hepatocytes and macrophages) and enters the 'endogenous' pathway of antigen processing. Alternatively, antigens of sporozoites, and perhaps of other microbes found in endocytic vesicles, may follow a different processing pathway. A better understanding of these mechanisms should facilitate the design of immunogens and vaccines which aim at inducing both antibodies and CD8⁺ cytotoxic T cells.

Our results are in agreement with earlier findings showing that cytotoxic-T-cell epitopes are contained within the CS protein of *P. falciparum*²¹. More important, we demonstrate that these epitopes can be the target of cell-mediated immunity, and that CD8⁺ T cells that are specific for a single epitope can confer protection against a parasitic infection. □

TABLE 2 Effect of passively transferred CS-specific cytotoxic T-cell clones on sporozoite challenge

Cells transferred* (no. cells $\times 10^6$)	Challenge†	No. protected/ total challenged	% Mice protected
Experiment 1			
C7 (18)	<i>P. berghei</i>	5/5	100
C1 (40)	<i>P. berghei</i>	16/21	76
B35‡ (30)	<i>P. berghei</i>	0/5	0
Normal spleen cells (40)	<i>P. berghei</i>	0/6	0
Experiment 2			
C11 (20)	<i>P. berghei</i>	5/5	100
Normal spleen cells (20)	<i>P. berghei</i>	0/5	0
Experiment 3			
C7 (25)	<i>P. berghei</i>	5/5	100
C7 (5)	<i>P. berghei</i>	0/5	0
Experiment 4			
C7 (20)	<i>P. berghei</i>	5/5	100
Normal spleen cells (20)	<i>P. berghei</i>	0/5	0
C7 (20)	<i>P. yoelii</i>	0/5	0
Normal spleen cells (20)	<i>P. yoelii</i>	0/5	0
Experiment 5			
C7 (20)	<i>P. berghei</i>	5/5	100
Normal spleen cells (20)	<i>P. berghei</i>	0/5	0
C7 (20)	<i>P. berghei</i> (blood forms)	0/5	0

* *P. berghei* CS-specific cytotoxic clones were maintained in culture by weekly re-stimulation as described²⁶. Groups of age-matched (8–10-week-old) female BALB/c mice were injected intravenously with each of the CTL clones collected 7 days after re-stimulation with antigen. As controls, other groups of mice were injected with normal spleen cells. Immediately before transfer, cells were resuspended in culture medium (0.5 ml) without serum and containing 10^3 U human recombinant interleukin-2. A second dose (10^3 U) of interleukin-2 was administered intraperitoneally 16 h after cell transfer. The mice were challenged 18 h after the cell transfer by intravenous injection of sporozoites. A final dose of 10^3 U interleukin-2²⁷ was administered intravenously 24 h after sporozoite challenge. Thin blood smears were prepared daily between day 3 and day 12 after challenge, stained with Giemsa and examined by light microscopy to detect parasitized red blood cells. Protection was defined as absence of parasitaemia.

† In experiments 1, 2, 3 and 4, the mice were challenged with 2,000 *P. berghei* sporozoites. In experiment 5 they were challenged either with 10,000 *P. berghei* sporozoites or with 2,000 infected red blood cells obtained from a *P. berghei*-infected mouse. In experiment 4, some groups of mice were challenged with 500 *P. yoelii* sporozoites.

‡ Mice injected with clones CS.B28 (17×10^6 cells per mouse), B83 (26×10^6) and CS.B35 (20×10^6) were not protected against sporozoite challenge. Partial protection, which needs to be confirmed in additional experiments, was obtained with three other clones (CS.C3, CS.B43 and CS.B18).

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An explanation for the protective effect of the MHC class II I-E molecule in murine diabetes

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INSULIN-DEPENDENT diabetes mellitus is widely believed to be an autoimmune disease¹. Recent onset diabetics show destruction of insulin-secreting pancreatic β -cells associated with a lymphocytic infiltrate (insulinitis)², with autoantibodies to β -cells being found even before the onset of symptoms³. Susceptibility to the disease is strongly influenced by major histocompatibility complex (MHC) class II polymorphism in both man⁴ and experimental animal models such as the non-obese diabetic (NOD) mouse⁵. As MHC class II molecules are usually associated with dominant immune responsiveness, it was surprising that introduction of a transgenic class II molecule, I-E, protected NOD mice from insulinitis and diabetes⁶. This could be explained by a change either in the target tissue or in the T cells presumed to be involved in β -cell destruction. Recently, several studies have shown that I-E molecules are associated with ontogenetic deletion of T cells bearing antigen/MHC receptors encoded in part by certain T-cell receptor $V\beta$ gene segments^{7–11}. To determine the mechanism of the protective effect of I-E, we have produced cloned $CD4^+$ and $CD8^+$ T-cell lines from islets of recently diabetic NOD mice. These cloned lines are islet-specific and pathogenic in both I-E⁺ and I-E[−] mice. Both $CD4^+$ and $CD8^+$ cloned T cells bear receptors encoded by a $V\beta 5$ gene segment, known to be deleted during development in I-E expressing mice¹⁰. Our data provide, therefore, an explanation for the puzzling effect of I-E on susceptibility to diabetes in NOD mice.

Studies of insulin-dependent diabetes mellitus (IDDM) in NOD mice, BioBreeding (BB) rats and man all demonstrate a pathogenic role for T cells, probably by direct cytotoxic effects on β -cells. In addition, it is clear that both $CD4^+$ and $CD8^+$ T cells are required to transfer diabetes to NOD mice^{12,13}. To further characterize the specific T cells involved in the immunopathogenesis of IDDM in NOD mice, we have isolated and cloned by limiting dilution a number of T-cell lines from the islets of recently diabetic NOD mice. Islet-specific cloned T-cell lines are propagated by feeding with NOD islets and exogenous interleukin-2 (IL-2). Among the cloned lines we have recovered, several respond specifically to NOD islets and not to NOD spleen cells (Fig. 1). The data shown in this paper all derive from a single $CD4^+$ and a single $CD8^+$ cloned T-cell line. Identical results have been obtained with three other $CD4^+$ T-cell lines and one other $CD8^+$ T-cell line (data not shown). Further analysis shows that the $CD4^+$ cloned T cells do not respond significantly to islets from BALB/c mice, differing from

NOD mice at many loci, including MHC. Unlike the one previously reported murine islet-specific cloned $CD4^+$ T-cell line¹⁴, the response of our $CD4^+$ cloned lines to islets did not require the addition of syngeneic feeder cells. $CD8^+$ cloned lines do respond to BALB/c islets; BALB/c and NOD are similar or identical at the class I MHC locus H-2K^d, perhaps accounting for this result⁵. B10.BR (H-2^k) islets stimulate neither cloned line significantly (data not shown). Thus, both $CD4^+$ and $CD8^+$ cloned lines appear to be islet-specific and MHC restricted. Because BALB/c mice are I-E positive, and because (NOD $\times$ BALB/c) F_1 hybrid mice develop neither insulinitis nor diabetes, which is consistent with the results in I-E transgenic NOD mice, we asked whether the putative islet autoantigens recognized by our cloned T-cell lines were expressed on these islets. As can be seen (Fig. 1), both $CD4^+$ and $CD8^+$ cloned T cells respond to (NOD $\times$ BALB/c) F_1 islets. This suggests that the protective effect of I-E expression is not due to an absence of stimulatory autoantigens on islet cells.

These cloned T-cell lines have been tested for their ability to transfer disease to sub-lethally irradiated young NOD mice, according to the protocol of Miller *et al.*¹³ for polyclonal T cells. As can be seen in Fig. 2a, neither $CD4^+$ nor $CD8^+$ clones injected alone cause significant insulinitis. As predicted by earlier studies using uncloned T cells^{12,13} and by injection of antibodies against CD4 (refs 15, 16) and CD8 (ref. 17), however, the mixture of islet-specific $CD4^+$ and islet-specific $CD8^+$ clones causes intense insulinitis by 28 days in most islets of all mice sampled. This mixture of cloned T cells causes loss of cells containing insulin immunoreactivity from islets invaded by lymphocytes in these mice (data not shown), as well as glycosuria on occasion. These results suggest that the transferred T cells markedly impair β -cell function. The fraction of cells found in the islets of such mice derived from the injected cloned T cells is not known. In parallel

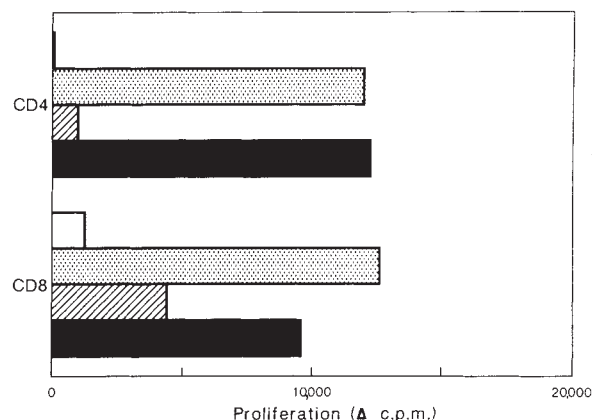


FIG. 1 NOD mouse islet derived $CD4^+$, $CD8^+$ and $CD8^+$, $CD4^+$ cloned T-cell lines demonstrate islet specificity and MHC restriction. Proliferation assays were used to determine the response of the T-cell clones using the following stimulators: NOD spleen cells (open bars), NOD islets (spotted bars), BALB/c islets (striped bars), and (NOD $\times$ BALB/c) F_1 islets (black bars). Note that both cloned lines respond equally to NOD islets (I-E⁺) and (NOD $\times$ BALB/c) F_1 islets (I-E⁺).

METHODS. Islet-specific T-cell clones were generated from islets obtained from newly diabetic NOD female mice. T cells were expanded in recombinant (r) IL-2 (10 U ml^{−1}) in Click's EHAA medium with 10% FCS. Every 21 days, rIL-2 was removed for 2 days. Cloned lines were stimulated with mitomycin-C treated NOD islets for two days, after which cells were expanded in rIL-2-containing medium. Cells were cloned by limiting dilution. Mouse islets were isolated by the method of Prowse *et al.*²⁹. In the proliferation assay, mitomycin C-treated splenocytes (10⁵) or islets (10) are cultured with islet specific T-cell clones (10⁴) in 0.2 ml Click's medium and 5% FCS in 96-well round-bottomed microtitre plates. After 48 h, rIL-2 (5 U ml^{−1}) is added to the wells, and at 96 h of total incubation, a terminal 16 h pulse with 1 μ Ci [³H]thymidine (6.7 Ci mmol^{−1}; ICN) was added to the cultures. The mean c.p.m. for thymidine incorporation are calculated from duplicate cultures.

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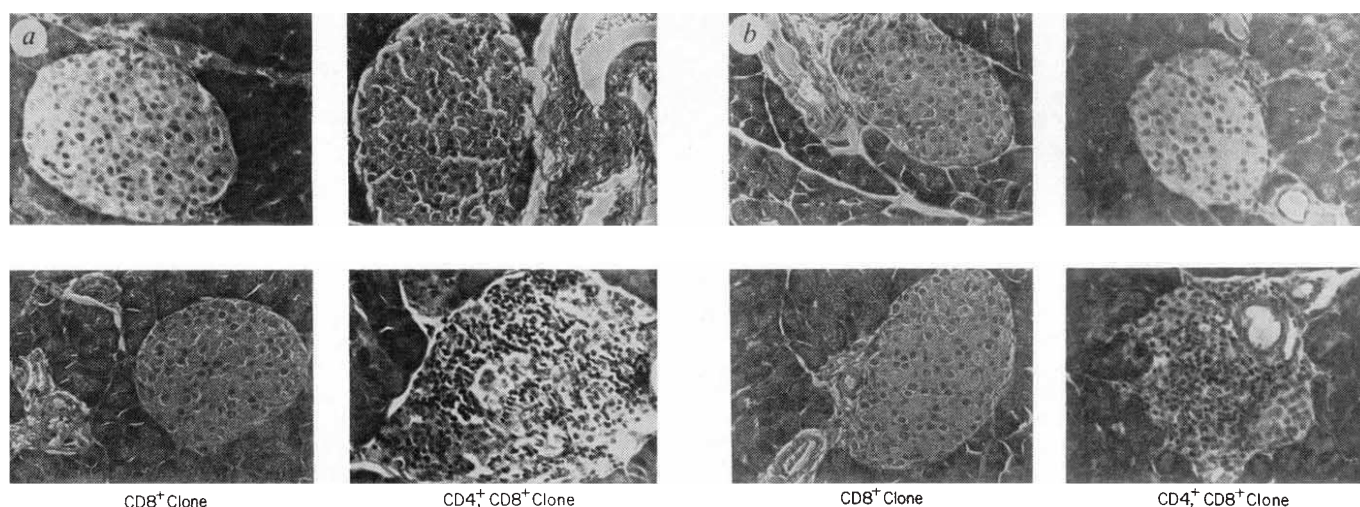


FIG. 2 A combination of a $CD4^+$ and a $CD8^+$ islet specific T-cell clone is required to produce insulitis in irradiated NOD (a) and (NOD $\times$ BALB/c)F1 (b) recipient mice. Representative pancreatic sections are shown from four treatment groups: saline (upper left), $CD4^+$ clone alone (upper right), $CD8^+$ clone alone (lower left), and a mixture of a $CD4^+$ and a $CD8^+$ clone (lower right). Insulitis, massive lymphocytic infiltration into the islet, is seen only in the groups that received a mixture of the two cloned lines.

studies, similar results are found in (NOD $\times$ BALB/c)F₁ irradiated recipients (Fig. 2b), again arguing against an effect of I-E on autoantigen expression in NOD islets. These studies also show that prior inflammation of the islets is not required to obtain stimulation of our cloned T-cell lines *in vivo* or *in vitro*. Thus, the responses of $CD4^+$ T cells are likely to be directed against secreted β -cell products processed and presented by antigen-presenting cells normally residing in the islet.

As I-E expression appears to be unable to protect islets from autoimmune attack by cloned, MHC-restricted, islet cell-specific T cells, we have examined an alternative hypothesis. It is now well established that expression of I-E is associated with the deletion during intrathymic ontogeny of T cells whose receptors are encoded by certain $V\beta$ genes^{7,10,11}. To determine if such deletional effects might explain the protective effect of I-E on diabetes in NOD mice, we determined the $V\beta$ genes expressed by the receptors on our cloned T-cell lines. As can be seen in Fig. 3, both the $CD4^+$ and the $CD8^+$ cloned T-cell lines express T-cell receptors that have β -chain variable regions which are partially encoded by $V\beta 5$; this was true of 3 of 3 other $CD4^+$ and 6 of 12 other $CD8^+$ cloned, islet-specific T-cell lines tested. It has recently been shown that T cells expressing $V\beta 5$ are ontogenetically deleted in I-E expressing mice¹⁰. Also, we have shown that NOD mice express $V\beta 5$ on ~5% of their T cells, whereas (NOD $\times$ BALB/c)F₁ mice express $V\beta 5$ on very few T cells (~1%). Finally, preliminary adoptive transfer experiments of splenic T cells from recently diabetic mice to irradiated young NOD recipients¹³ gives diabetes in all recipients, whereas depletion of $V\beta 5$ -bearing T cells from this population gives almost no diabetes (E.-P.R., O.K., R.S.S. and C.A.J., unpublished data). Thus, if the cloned, pathogenic T-cell lines we have isolated are representative of the cells involved in β -cell destruction in NOD mice, as seems likely from the above studies, then one can understand the protective effect of I-E.

Two other issues are worth noting. First, the mechanism by which T cells, distinguishable only by expression of the T-cell antigen receptor (TCR) encoded by particular $V\beta$ gene segments, are deleted by I-E molecules is unclear. However, it is probable that it is not I-E alone which is responsible for the deletional effects, as similar effects are observed in deletion by *Mls* locus polymorphisms^{8,9}. Although the precise nature of the

METHODS. Female NOD and (NOD $\times$ BALB/c)F1 mice, 7–8 weeks old, were given whole-body irradiation (700 rads) before being injected intravenously with 5×10^6 cloned T cells in 0.3 ml saline. Recipient mice were killed 4 weeks after treatment for histopathology. The tissue was fixed in buffered formalin, paraffin-embedded, sectioned and stained with haematoxylin and eosin.

Mls locus product is unknown, a highly analogous situation is observed with certain bacterial proteins, such as the *Staphylococcal* enterotoxins^{18–20} (J. Yagi and C.A.J., manuscript submitted). These proteins seem to act by binding to the outer face of the class II molecule and to the outer face of the $V\beta$ -encoded segment of the TCR (ref. 19). It has also been shown that T cells expressing a receptor encoded by $V\beta 17a$ do not respond to I-E expressed on transfected L cells, although they do respond

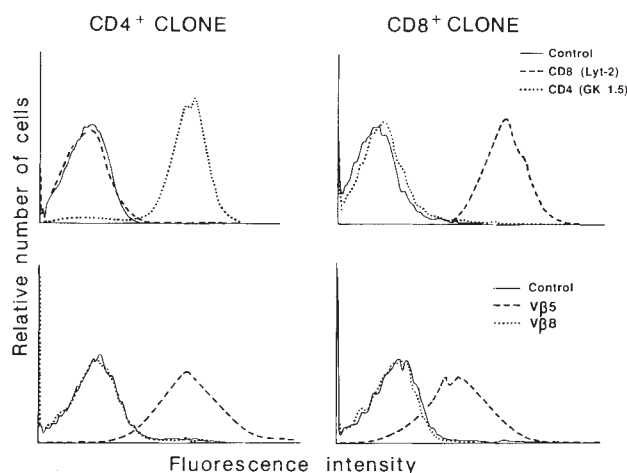


FIG. 3 Both $CD4^+$, $CD8^-$ and $CD8^+$, $CD4^-$ islet specific T-cell clones express T-cell receptors with β -chain variable regions which are encoded by $V\beta 5$. Immunofluorescent analysis was carried out using the following biotin-conjugated monoclonal antibodies: Controls, FITC-Avidin alone; upper panels, anti- $CD8$, anti- $CD4$; and lower panels, anti- $V\beta 5$ (MR9-4) (O.K. & E. Palmer, manuscript submitted) and anti- $V\beta 8$ (F23.1) (ref. 30).

METHODS. Biotin-conjugated antibodies, at optimum concentrations, were added to 10^6 T cells for 30 min at 4 °C in phosphate-buffered saline containing 2% FCS and 0.1% sodium azide (staining buffer). After washing with staining buffer, cells were stained with FITC-Avidin for 30 min at 4 °C. Washed cells were analysed on a FACS 440. Cell number is displayed against a 3.7 log unit axis of fluorescence intensity.

to I-E expressed on B cells²¹. Such cells are ontogenetically deleted in I-E positive mice. These studies suggest that all such deletion events may involve *Mls*-like molecules. Presumably the I-E molecules expressed in NOD transgenic mice delete V β 5-bearing cells by such a mechanism and thus protect the animal from autoimmune β -cell attack.

The second issue involves the apparent protective effect of certain HLA-DR alleles in human IDDM. It is well known that HLA-DR2 is negatively associated with IDDM in man²². Could this class II molecule be deleting potentially autoreactive T cells? This question is worth examining, even though current evidence indicates that V β deletion is not a common event in man²³. Rather, it has been argued that the protective effect of certain HLA-DR alleles is due to linkage disequilibrium with a non-susceptible allele at HLA-DQ (refs 4, 24). This raises an alternative intriguing possibility, namely, that linkage disequilibrium in HLA is maintained so that the deletion of certain TCRs in people of each HLA-DR genotype leaves residual TCRs that can interact effectively with the linked HLA molecules to generate protective immunity. It is worth noting that the protective effect of HLA-DR2 is seen in heterozygotes as well, indicating that this effect is dominant. Deletion of potentially autoreactive T cells in such individuals is, therefore, a possibility.

One can argue that autoimmune diseases are diseases of receptor repertoire, in which lymphocytes bearing autoreactive receptors are activated and cause tissue damage. The studies reported here are consistent with this proposal. Similarly, recent studies have shown a remarkable homogeneity in autoreactive T cells in experimental allergic encephalomyelitis^{25,26}, an induced autoimmune disease of mice, and in rheumatoid arthritis, a spontaneous autoimmune disease in man²⁷. That diabetes may fall into this same group further supports the notion that the outgrowth of 'forbidden clones' may underlie many such diseases²⁸. This raises the hope that anti-receptor immunotherapy may be more beneficial than would be predicted on the basis of effects of anti-idiotypic antibody administration in conventional immune responses. □

Myosin I is located at the leading edges of locomoting *Dictyostelium amoebae*

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MOVEMENT of a eukaryotic cell along a substrate occurs by extension of lamellipodia and pseudopodia at the anterior and retraction at the posterior of the cell. The molecular and structural mechanisms of these movements are uncertain. *Dictyostelium discoideum* contains two forms of myosin. Here we show by immunofluorescence microscopy that non-filamentous myosin I occurs at the leading edges of the lamellipodial projections of migrating *Dictyostelium amoebae*, which are devoid of myosin II, whereas filamentous myosin II is concentrated in the posterior of the cells. On the basis of these locations of the two forms of myosin and their known biochemical and biophysical properties, we suggest that actomyosin I may contribute to the forces that cause extension at the leading edge of a motile cell, while the contraction of actomyosin II at the rear squeezes the cell mass forward. Myosin I isozymes might have similar roles in metazoan cells, for example at the leading edges of neuronal growth cones, and in the extension

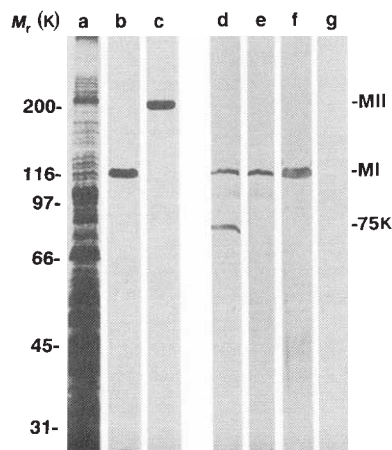


FIG. 1 Lanes a–c, Coomassie blue-stained gels of total *Dictyostelium* cell lysate (lane a), purified myosin I (lane b) and purified myosin II (lane c). Lane d, Immunoblot of a cell lysate probed with DM1, which recognizes peptides of 117K (myosin I, heavy chain) and 75K. Lane e, Immunoblot of a cell lysate probed with pDM1 (DM1 after adsorption with partially purified 75K protein). Lanes f and g, Immunoblots of purified myosin I (lane f) and myosin II (lane g) with pDM1. M_r s for migrating standard proteins are indicated (in thousands) on the left; positions of myosin II (MII) and myosin I (MI) heavy chains, and 75K protein are shown on the right.

METHODS. Myosin I was purified from *D. discoideum* (strain AX-3) essentially as described for *Acanthamoeba* myosin I²⁰. Purified myosin II²² was a gift from E. Kuczmarski. The 75K polypeptide was partially purified by chromatography on DE-52 (Whatman), hydroxyapatite (BioRad), and MonoQ (Pharmacia). A New Zealand white rabbit was immunized by two injections, each containing 200 μ g myosin I in Freund's adjuvant and spaced three weeks apart, and was bled 10 days after the second injection. The antiserum so obtained (DM1) was adsorbed (pDM1) with the 75K polypeptide immobilized on nitrocellulose membranes. After separation SDS-PAGE²³ and transfer to nitrocellulose membranes²⁴, the proteins were immunoblotted. Nitrocellulose replicas were incubated in DM1 and pDM1 diluted 800-fold. Primary antibodies were visualized after incubation with peroxidase-conjugated goat anti-rabbit IgG (Boehringer Mannheim).

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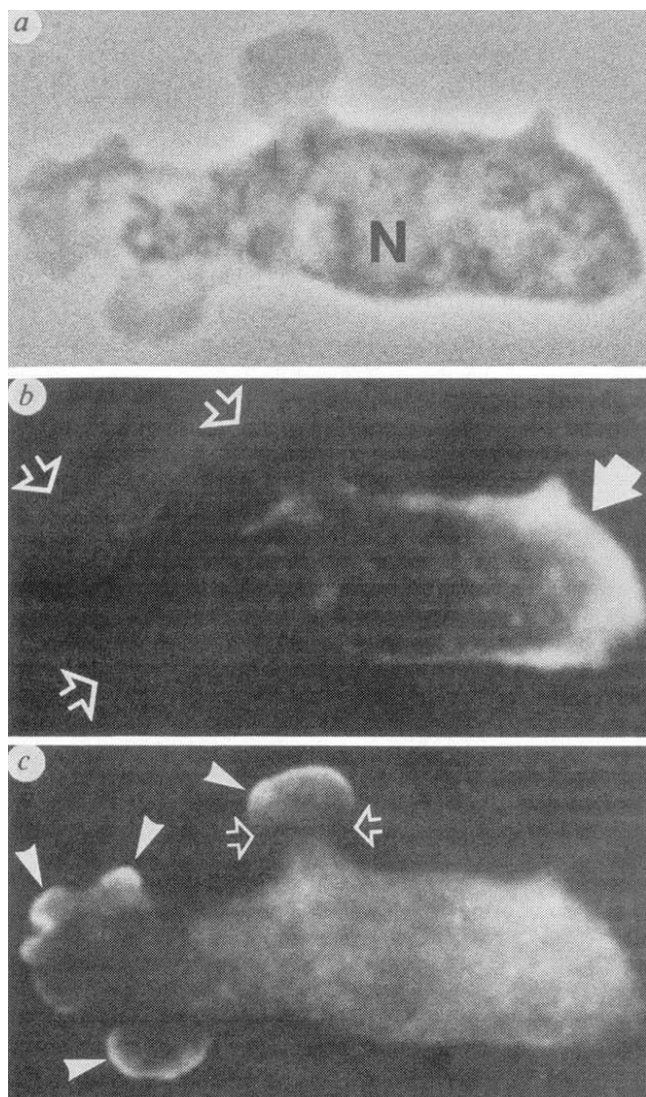


FIG. 2 Differential localization of myosin I and myosin II in a migrating *Dictyostelium* amoeba. *a*, Phase-contrast image of a representative cell migrating from right to left. The cell has formed one lamellipodium and two pseudopodia. *N*, denotes the nucleus. *b* and *c*, Immunofluorescence micrographs of the same cell stained for myosin II (*b*) and for myosin I (*c*). Magnification, $\times 3,450$.

METHODS. *D. discoideum* amoebae (strain NC4) were plated on coverslips and prepared for immunofluorescence by the 'agar-overlay' technique¹⁰. Briefly, samples were fixed for 5 min at -15°C with 1% formaldehyde in absolute methanol and washed with PBS solution (137 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4 , 8.0 mM Na_2HPO_4 , pH 7.3) for 10 min. After removing the agarose, samples were incubated for 30 min at 37°C in a 1:1 mixture of pDM1 (rabbit anti-myosin I) diluted 1:80 in PBS, and DM2 (mouse monoclonal IgG against myosin II) at $2\text{ }\mu\text{g ml}^{-1}$ in PBS. After washing with PBS for 10 min, the sample was stained for 30 min at 37°C with a mixture of fluorescein isothiocyanate-labelled goat anti-rabbit IgG, F(ab')₂ (Sigma) and tetramethylrhodamine isothiocyanate-labelled goat anti-mouse IgG (Southern Biotechnology Associates), both diluted 1:25 (final) in PBS. Non-specific staining was eliminated by carefully selecting among the commercially obtained second antibodies and by pre-adsorbing them with cell lysate¹⁴. Samples were washed with PBS, mounted in a mixture of 10% (w/v) 1,4-diazabicyclo[2,2,2]octane, 30% glycerol and 20% polyvinylalcohol in PBS¹⁴, and observed under a Zeiss Photomicroscope III equipped with Plan Neofluar $\times 40$ (N.A. 0.9), Plan Apo $\times 63$ (N.A. 1.4) and Neofluar $\times 100$ (N.A. 1.3) objective lenses. Micrographs were taken on Tmax-400 film (Kodak) developed with Diafine (Acufine).

of lamellipodia and pseudopodia of leukocytes, macrophages and fibroblasts.

Dictyostelium amoebae, which chemotax as elongate, polarized cells, provide a favourable system for studying the role of actomyosin in cell motility¹. Actin^{2,3} and two forms of myosin have been purified from *Dictyostelium*: myosin II (ref. 4) is a conventional myosin of native relative molecular mass (M_r) $\sim 550,000$ (550K) that self-assembles into bipolar filaments analogous to the thick filaments of the skeletal muscle sarcomere. Myosin I (ref. 5) by contrast, is a single-headed globular protein of $M_r \sim 140\text{K}$ which is incapable of forming filaments. It is very similar to the much more extensively characterized myosin I isoforms from *Acanthamoeba*⁶. Both myosins I and II are mechanochemical enzymes which generate force through the hydrolysis of ATP when complexed with F-actin⁶.

Recently two genetically engineered myosin II-deficient strains of *Dictyostelium*^{7,8} were found to be defective in cytokinesis, but capable of phagocytosis and limited chemotaxis.

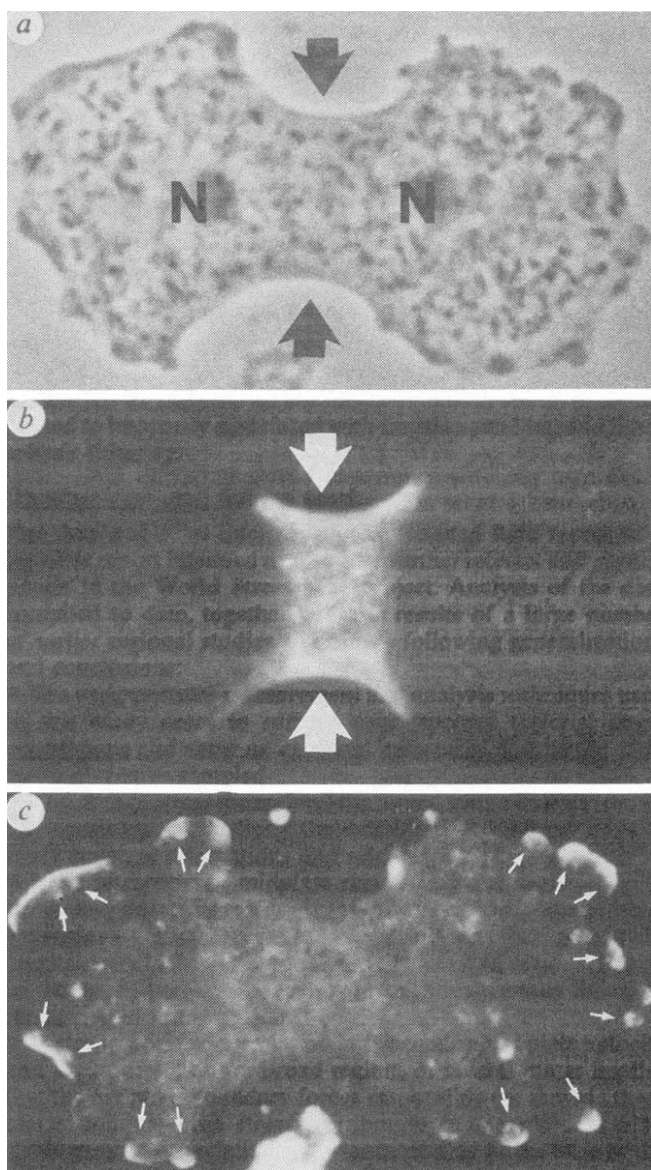


FIG. 3 *Dictyostelium* amoeba during cytokinesis. *a*, Phase-contrast micrograph of a cell with a typical cleavage furrow (arrows) and surface projections at its poles. *N*, denotes nuclei. *b* and *c*, Immunofluorescence micrograph of the same cell stained for myosin II (panel *b*) and for myosin I (panel *c*). Magnification, $\times 3,800$.

The velocity of amoeboid movement of these cells is substantially impaired, but the rate of formation of pseudopodia is relatively normal⁹, although the pseudopodial area and growth rate are diminished. These results indicate that myosin II is required for normal translocation but is not essential for phagocytosis or the extension of pseudopodia at the leading edge. We were therefore interested to investigate the intracellular distribution of myosin I in normal motile cells.

We used two antibodies in the studies illustrated here. DM2, a monoclonal antibody against myosin II (ref. 10), recognizes only the myosin II heavy chain in immunoblots of total cell extracts and does not react even with large amounts of purified myosin I (data not shown). DM1, the antiserum raised against purified *Dictyostelium* myosin I, recognizes two polypeptides in immunoblots of total cell lysates, the 117K myosin I heavy chain and an unidentified 75K polypeptide (Fig. 1, lane *d*). Specific anti-myosin I serum (pDMI) (Fig. 1, lane *e*) was obtained by adsorption of DM1 with a partially purified preparation of the 75K polypeptide. We found that pDMI does not recognize myosin II on immunoblots of cell lysates or of the purified protein (Fig. 1, lanes *e* and *g*).

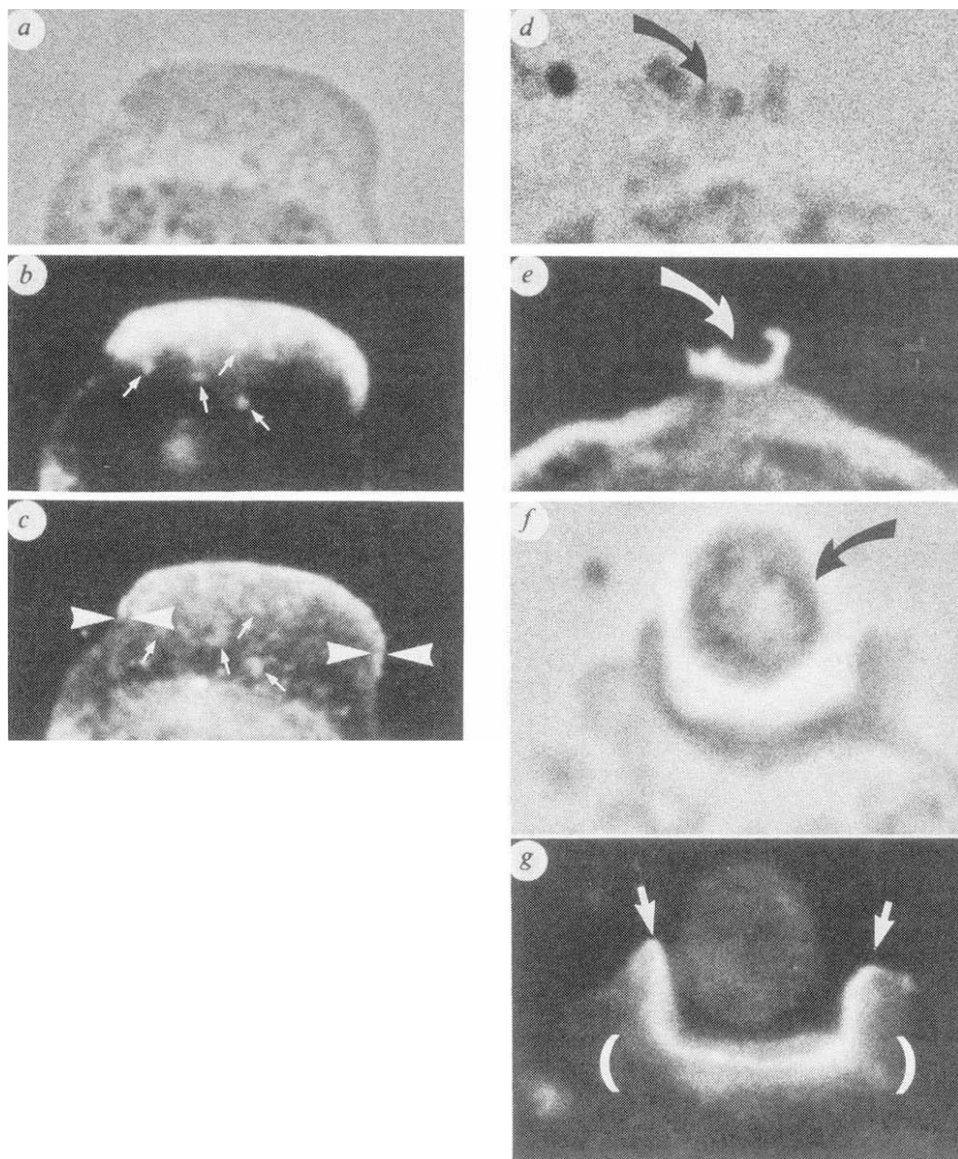
Figure 2*a* shows a phase-contrast image of a typical migrating cell. As we expected from earlier work¹⁰⁻¹², myosin II is concen-

trated in the posterior cortical region (Fig. 2*b*; large arrow). A limited number of myosin filaments are scattered throughout the cytoplasm, but essentially no myosin II is detected in the lamellipodia or pseudopodia (Fig. 2*b*; open arrows). In marked contrast, myosin I is concentrated in the submembranous cortex at the leading edge of lamellipodia and pseudopodia (Fig. 2*c*; arrowheads). The intensity of staining of myosin I, and the distance it extends in from the margin of the cell, varies in different projections, possibly reflecting the dynamic nature of the anterior of a migrating amoeba. A substantial amount of myosin I is also diffusely distributed in the cytoplasm, particularly in the posterior of the cells, but regions behind the leading edge contain little or no myosin I (Fig. 2*c*; open arrows).

Results were identical when we used two antisera raised against *Acanthamoeba* myosin I and a second antiserum against *Dictyostelium* myosin I. Each of these four antisera reacts strongly with myosin I and very weakly with different patterns of lower molecular weight peptides on immunoblots of total cell lysates; other bands are almost undetectable with the second *Dictyostelium* myosin I antiserum, and adsorption with total amoeba protein minus myosin I has no effect on the immunoblots. Cells did not stain with pre-immune serum or with the second antibodies alone (goat anti-rabbit

FIG. 4 Localization of myosin I at the leading edge. *a*, Phase-contrast and *b* *c*, fluorescence micrographs of the same lamellipodium stained for *F-actin* (*b*) and myosin I (panel *c*). Magnification, $\times 10,000$. *d* and *e*, Localization of *F-actin* in the phagocytic cup. *d*, Phase-contrast micrograph of a phagocytic cup internalizing bacteria (arrow). *e*, Fluorescence micrograph of the same region stained for *F-actin*. Magnification, $\times 6,000$. *f* and *g*, Localization of myosin I in the phagocytic cup. *f*, Phase-contrast micrograph of a phagocytic cup of one amoeba internalizing what appears to be a fragment of another amoeba (arrow). *g*, Immunofluorescence micrograph of the same region stained for myosin I. Magnification, $\times 6,700$.

METHODS. Sample preparation and microscopy were as described in the legend to Fig. 2, except that the sample shown in *a-c* was incubated for 20 min at 37 °C with rhodamine-phalloidin (Molecular Probes), diluted 1:5 in PBS after staining with pDMI and its secondary antibody; also, the sample shown in *d* and *e* was stained with only rhodamine-phalloidin.



and goat anti-mouse IgG). Each of the images in Figs 2–4 is characteristic of more than 1,000 cells and we confirmed these observations by through-focus images of cells that had not been flattened.

Amoebae undergoing cytokinesis form a cleavage furrow and distinctive local pseudopodial projections (Fig. 3a). As before^{10,12}, myosin II is concentrated in the cleavage furrow of the dividing cell (Fig. 3b; arrows). Myosin I does not accumulate in the cleavage furrow (Fig. 3c) but is concentrated in the pseudopodia at the two poles of the dividing cell (Fig. 3c; small arrows). The diffuse cytoplasmic fluorescence of myosin I is less intense in the polar regions of dividing cells, which may correspond to the exclusion of myosin I from the regions behind the leading edge of migrating amoebae.

Fluorescence localization of F-actin with rhodamine-labelled phalloidin confirms earlier reports^{10,13,14} of its existence in the lamellipodia and pseudopodia projecting from the anterior of the cells and in the posterior cortical regions (data not shown). Interestingly, double fluorescence staining of lamellipodia of migrating cells (Fig. 4a) shows that myosin I is, for the most part, restricted to a narrow zone at the leading edge (Fig. 4c), whereas F-actin occupies a broader zone extending further into the body of the cell (Fig. 4b). Small foci behind the leading edge stain for both F-actin and myosin I (Fig. 4b and c; small arrows).

Formation of a cortical projection is an early step in the ingestion of particles by these amoebae. The 'phagocytic cup' contains little or no myosin II (ref. 10). We find that the phagocytic cup stains strongly for F-actin (Fig. 4e) and myosin I (Fig. 4g). Myosin I occupies a zone ~1 µm wide beneath the cup (Fig. 4g; parentheses) and is most concentrated in a 300-nm band immediately below the plasma membrane (Fig. 4g; arrows).

Our data establish that, in *Dictyostelium*, myosin I is present in lamellipodia, pseudopodia and phagocytic cups, which are regions containing well developed networks of actin filaments but essentially no myosin II. By contrast, myosin II is concentrated in the posterior cortex of migrating amoebae and in the contractile ring of dividing cells, which are regions not enriched in myosin I. We cannot determine by immunofluorescence microscopy whether the myosin I in the leading edge of the *Dictyostelium* amoebae is associated with the plasma membrane or is embedded in the cortical actin network, or both, but a substantial fraction of *Acanthamoeba* myosin I is known to be tightly associated with the plasma membrane^{15,16}.

These observations suggest that actomyosin I-dependent force-generating activity occurs at the leading edge (as in pseudopodia extension) and that actomyosin II-dependent force-generating activity occurs at the trailing end of a migrating *Dictyostelium* amoeba (causing the cell mass to move forwards). This could explain how myosin II-minus mutants can form smaller-than-normal pseudopodia at a relatively normal rate⁹. The myosin I that is more generally distributed in the cytoplasm might be involved in other motile activities¹⁶, perhaps with myosin II; or it might represent, at least in part, the enzymatically inactive (unphosphorylated) state of myosin I (refs 5 and 6). The conventional sliding-filament model is probably adequate to explain the contractile activity of actomyosin II, but the mechanism by which myosin I associated with the plasma membrane and F-actin could generate an extensive force is uncertain. Membrane-bound *Acanthamoeba* myosin I can generate force against actin cables however¹⁷, and both *Acanthamoeba* and *Dictyostelium* myosin I will crosslink actin filaments and generate force between crosslinked filaments^{18–21}.

None of our observations is incompatible with the participation either of other processes in amoeboid movement, such as membrane flow or the remodelling of the actin matrix, or of myosin I and myosin II in other motile activities. The significance of our results is that they show the presence in the leading edge of a migrating cell of myosin I, which in conjunc-

tion with F-actin, is known to be capable of producing force and movement. □

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Sequence-specific DNA-binding activities of the gap proteins encoded by *hunchback* and *Krüppel* in *Drosophila*

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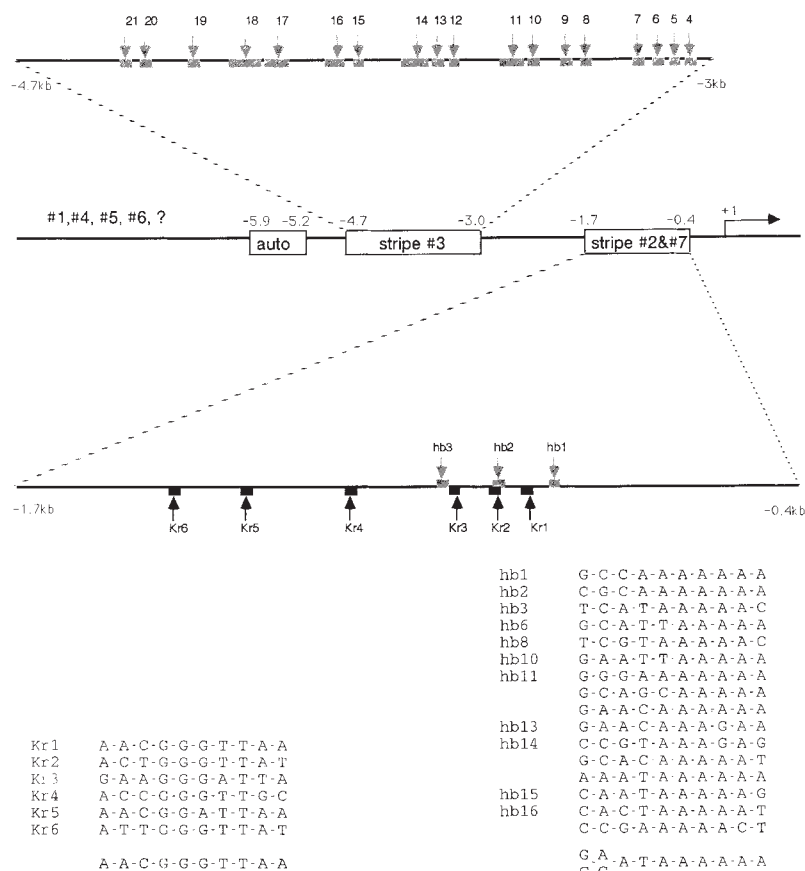
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THE segmentation of the *Drosophila* body plan depends on a hierarchy of interactions among ~20–25 regulatory genes that are active in the early embryo (refs 1–4; for a review see ref. 5). The gap genes have a key role in this process and are responsible for the periodic expression of certain pair-rule genes^{6–8} and the localized expression of several homoeotic genes^{9–11}. The two best characterized gap genes, *hunchback* (*hb*) and *Krüppel* (*Kr*), contain homologies with the zinc-finger DNA-binding motif^{12,13}, although their mode of action in the early embryo is unknown. Here we report that both of the proteins encoded by these genes possess sequence-specific DNA-binding activities, which indicates that they might regulate gene expression at the level of transcription. The binding sites of the *hb* gene product are related by a 10-base pair (bp) consensus sequence, $\frac{G}{C}/\text{ATAAAAAA}$, whereas the binding sites of the *Kr* gene product share a distinct 10-bp motif, AACGGGTAA. It is possible that the *hb* and *Kr* proteins co-operatively regulate gene expression, because they are expressed in broad, overlapping gradients in the early embryo. We also provide evidence that the on/off periodicity of the pair-rule gene *even-skipped* (*eve*)^{14,15} involves the interaction of the *hb* and *Kr* proteins with defined *eve* promoter elements^{16,17}.

We found that full-length *hb*- and *Kr*-encoded proteins produced in *Escherichia coli* are able to bind to specific sequences in the *eve* promoter. Our choice of *eve* for this analysis was based on previous genetic circuitry studies, which indicate that *eve* is an 'early' pair-rule gene that is directly regulated by the gap genes^{8,17,18}. Promoter-fusion studies have identified regions

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FIG. 1 Hb and Kr sites in the *eve* promoter. The central horizontal line shows the three *cis*-regulatory elements in the *eve* promoter identified. The arrow indicates the transcription start site (+1)^{16,17}. The initiation of stripe 3 depends on sequences located between -4.7 and -3 kb. Truncated promoters, or promoters carrying internal deletions, that disrupt this region fail to initiate stripe 3. The initiation of stripes 2 and 7 depends on sequences located between -1.7 and -0.4 kb. Sequences located between -5.9 and -5.2 kb mediate *eve* autoregulation (Auto) during later stages of development. The regulatory sequences responsible for the initiation of stripes 1, 4, 5 and 6 were not identified in previous studies, and seem to map further upstream than -8 kb. Stripe-2 and stripe-3 elements were scanned for Hb and Kr sites by DNaseI footprint assays using overlapping DNA fragments which spanned both promoter elements. Regions protected by the *hb* protein are indicated in grey, whereas Kr sites are indicated in black. The stripe-3 promoter element (above) contains 18 Hb sites, but does not contain any high affinity Kr sites. The stripe-2 element (below) contains six Kr sites and three Hb sites. Note that each Hb site in the stripe-2 element is closely linked to a Kr site. All six Kr sites are related by a 10-bp consensus sequence shown in the bottom left column. Alignment of the Hb sites reveals a distinct consensus sequence, shown in the bottom right column. Because of lack of space, not all of the Hb sites are listed, although all contain at least one copy of the consensus sequence. Note that some of the Hb sites contain multiple copies of the consensus sequence. For example, the Hb14 site contains three copies. The boundaries of the stripe-2 and -3 elements are based on studies by Harding *et al.*¹⁶. An independent study was done by Goto *et al.*¹⁷, which included an examination of *eve* promoter fusions carrying internal deletions. This study identified narrower limits for the elements essential for initiation of the two stripes. For example, Goto *et al.*¹⁷ propose that the stripe-3 element resides between -3.8 and -3 kb, which indicates that the Hb15-Hb21 sites might not be critical for the initiation of stripe-3. Perhaps the multiple Hb sites in the stripe-3 element are functionally redundant. Alternatively, optimal expression of stripe 3 might depend on all of the Hb sites, whereas the initiation of the stripe involves just a subset of the sites. Similar arguments pertain to the stripe-2 element. Goto *et al.*¹⁷ propose that this element maps between -1.7 and -1.1 kb, which indicates that the sites Hb1, Hb2, Kr1 and Kr2 are not essential for the initiation of stripe 2. Unfortunately, it is not clear whether stripe 2 is expressed at optimal levels when driven by *eve* promoters that lack sequences between -1.1 and -0.4 kb. Interestingly, an *eve* promoter lacking a 480-bp region in the stripe-2 element, which deletes the sites Hb3, Kr3, Kr4 and Kr5, fails to initiate stripe 2. This observation is consistent with the notion that one or more of these binding sites may be important for the expression of stripe 2. More rigorous evidence for this possibility depends on



in the *eve* promoter that are essential for the initiation of stripes 2, 3 and 7, and are probable targets for *hb* and *Kr* activity^{16,17} (see Fig. 1).

By using DNaseI footprint assays, we systematically searched for sites able to bind the *hb* or *Kr* protein (termed Hb or Kr sites, respectively) in overlapping DNA fragments that encompassed the *eve* promoter elements essential for stripes 2 and 3 (termed the stripe-2 and stripe-3 elements, respectively). The results of such an analysis for the stripe-2 element are shown in Fig. 2. A 280-bp *HinfI*-*FspI* fragment from the stripe-2 element was labelled with ³²P, incubated with increasing amounts of *hb* or *Kr* protein extracts, and then partially digested with DNaseI. This fragment contains three Kr sites (Kr1, Kr2 and Kr3) and three Hb sites (Hb1, Hb2 and Hb3), which represent most of the binding sites present in the stripe-2 element (Fig. 1). The proteins encoded by *hb* and *Kr* seem to recognize distinct sequences. An increase in the concentration of *hb* protein resulted in the protection of a sequence located near the labelled end of the DNA fragment (the Hb1 site; Fig. 2, lanes 5-7), which was not bound by the *Kr* protein present at any of the concentrations tested in this assay (Fig. 2, lane 4). Conversely, the *Kr* protein gave strong protection of a sequence (Kr1) that was not recognized by the *hb* protein. Despite their recognition of distinct sequences, some of the Kr and Hb sites are closely linked. For example, within the resolution of DNaseI

evaluating the *in vivo* expression of otherwise normal *eve*-promoter fusions carrying point mutations in the binding sites.

METHODS. Full-length *hb* and *Kr* proteins were prepared with the T7 expression vector pAR3040 (ref. 27), similar to a previously described method²⁸. *In vitro* mutagenesis was done to create an *NdeI* site at the translation initiation site for each coding sequence, and each sequence was then cloned into the unique *NdeI* site of the vector. Proteins were expressed in the bacterial strain BL21(DE3), and ~15-30% of the total protein obtained after induction with IPTG corresponds to *hb* or *Kr* protein. Total induced extract was solubilized in buffer Z (ref. 28) containing 4 M urea.

protection, the Kr2 and Hb2 sites seem to overlap, as do the Kr3 and Hb3 sites. The six Kr sites that were identified within the stripe-2 element are related by a 10-bp consensus sequence AACGGGTAA (Fig. 1).

Systematic searches of the stripe-3 element failed to reveal any high-affinity Kr sites, although it was shown to contain nearly 20 different Hb sites. Examples of the footprint assays done with stripe-3 sequences are shown in Fig. 3. Both of the DNA fragments shown contain several regions that were protected after addition of high concentrations of *hb* protein. These regions contain at least one copy of a 10-bp consensus sequence, G_A/C_AATAAAAAA (Fig. 1). Several of the binding sites (such as Hb18) contain as many as four copies of this consensus (Fig. 3b, vertical arrows).

The patterns of *hb* and *Kr* expression in the early embryo indicate that the *hb* and *Kr* proteins could function cooperatively to regulate gene expression. Double-staining studies with mixtures of antibodies directed against the *hb* and *Kr* proteins showed that the two proteins are distributed in broad gradients, which overlap extensively (Fig. 4a). The anterior domain of *hb* expression (shown in red) overlaps with the *Kr* expression domain (shown in green). The region of the overlap stained yellow (indicated by arrows) and includes a transverse stripe of at least eight cells in width that encircles the embryo. Overlap in the *hb* and *Kr* expression patterns is observed from the time

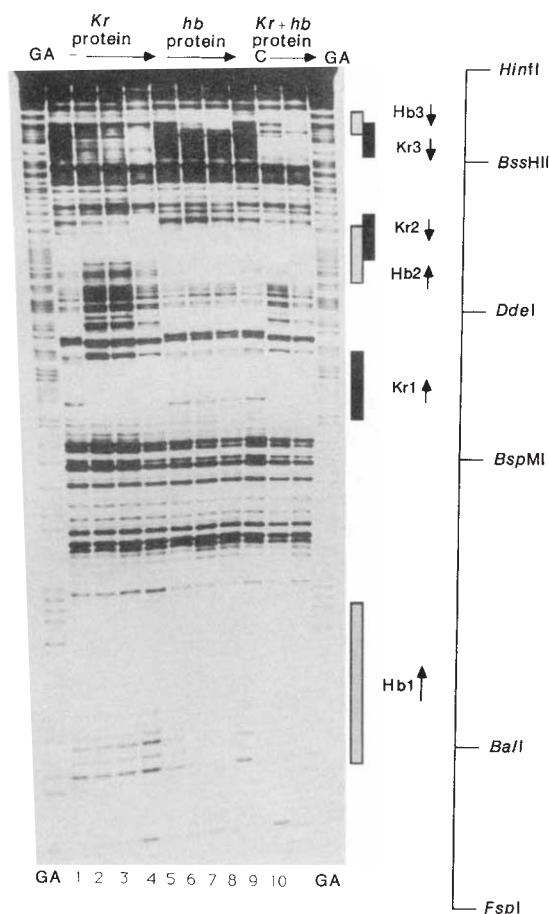


FIG. 2 Hb and Kr sites in the stripe-2 element. A 280-bp *HinfI*-*FspI* fragment from the stripe-2 element was labelled with ^{32}P at the *FspI* site and incubated with increasing amounts of *Kr* and *hb* protein extracts. Lanes 2-4, 25, 50 and 75 μg , respectively, of *Kr*-protein extract. There are three regions that were protected from digestion with DNaseI, Kr1, Kr2 and Kr3 (black rectangles on right). Lanes 5-7, 25, 75 and 150 μg , respectively, of *hb*-protein extract. Three regions of protection are observed, Hb1, Hb2 and Hb3 (grey rectangles on right). Vertical arrows indicate orientations of consensus sequences. Lanes 9 and 10, footprint assays done with mixtures of the *hb* and *Kr* encoded protein extracts. Lane 9, 25 μg *hb*-protein extract and 25 μg *Kr*-protein extract. Lane 10, 75 μg *hb*-protein extract and 75 μg *Kr*-protein extract. Mixing experiments were done by adding both proteins simultaneously. Lane 1 (—), negative control in which DNaseI digestion of the *HinfI*-*FspI* fragment was done without added protein. Lane 8 (C), control done with 75 μg protein extract from cells containing the T7 expression vector without the *hb* or *Kr* cDNA inserts. Lanes labelled GA, deoxyguanosine and deoxyadenosine sequences of the *HinfI*-*FspI* DNA fragment.

METHODS. DNaseI-protection assays were carried out essentially as previously described^{28,29}. Binding reactions were done with 2-5 ng ^{32}P -labelled DNA and 0.5 μg poly(dI) poly(dC) and 0.25 μg sonicated salmon-sperm DNA in 50 μl buffer 2 for 90 min on ice. Bacterial *hb* and *Kr* proteins are extremely insoluble (particularly *hb* protein), and required the use of urea as a solvent. Urea-extracted proteins were stored at -80°C and small aliquots were added directly to the binding reactions. Two different binding buffers were used, both providing similar results. Buffer 1: 110 mM KCl, 47.5 mM HEPES buffer, pH 7.9, 13.75 mM MgCl_2 , 1 mM DTT, 0.1 mM ZnCl_2 , 12% glycerol and 0.06% Nonidet P-40 detergent. Buffer 2: 100 mM KCl, 35 mM HEPES buffer, pH 7.9, 9.5 mM MgCl_2 , 0.1 mM EGTA, 1.6 mM DTT, 0.06 mM ZnCl_2 , 12% glycerol and 0.06% Nonidet P-40 detergent. After binding, 50 μl of 10 mM MgCl_2 /5 mM CaCl_2 were added to the reaction, and then 3.5 μl freshly diluted DNaseI (Worthington) to give a final concentration of 10 $\mu\text{g ml}^{-1}$. DNaseI digestion was stopped after 2-5 min on ice by addition of 90 μl of 1% SDS, 20 mM EDTA, 200 mM KCl, and 250 $\mu\text{g ml}^{-1}$ yeast transfer RNA. The samples were extracted with phenol, phenol-chloroform (1:1), chloroform, and then ethanol precipitated and electrophoresed in an 8% polyacrylamide/7.5M urea gel.

of the initial detection of the *hb* and *Kr* proteins, and persists during gastrulation (data not shown). This overlap is far more extensive than that indicated by previous localization studies¹⁹⁻²¹, and might have important implications for the way in which gap genes specify striped patterns of gene expression (see below). The limits of the *hb* and *Kr* expression patterns were further mapped in double-staining studies using antibodies directed against the *eve* protein. The *Kr* expression domain extends from the posterior margin of *eve* stripe 2 to the anterior margin of stripe 5 (Fig. 4b), whereas the anterior *hb* expression domain encompasses *eve* stripes 1 and 2 and extends into the

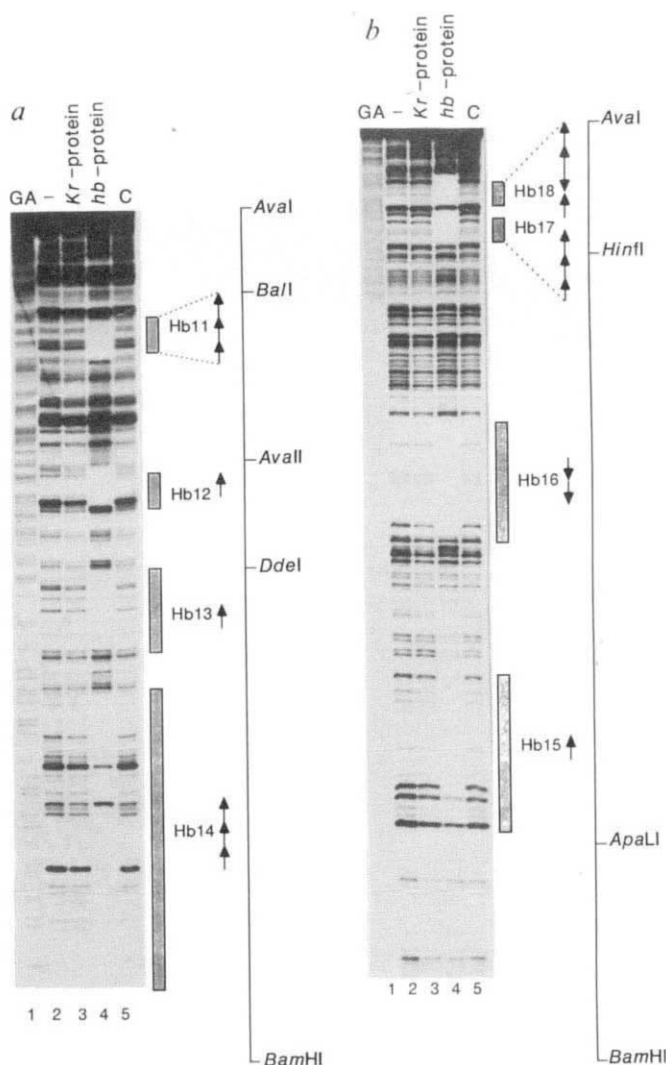


FIG. 3 Hb sites in the stripe-3 element. a, A 511-bp *BamHI*-*Aval* fragment from the stripe-3 element was ^{32}P -labelled at the *BamHI* site and incubated with 75 μg *Kr*-protein extract (lane 3) and 75 μg *hb*-protein extract (lane 4). The *Kr* extract failed to bind any sites in this fragment. Four of the protected regions obtained with the *hb* extract are indicated by the rectangles on the right. The vertical arrows indicate the orientations of a 10-bp consensus sequence in each site (see Fig. 1). A fifth Hb site occurs near the top of the gel. Note the appearance of hypersensitive sites that flank each of the binding sites. b, A 583-bp *Aval*-*BamHI* fragment from the stripe-3 element was ^{32}P -labelled at the *BamHI* site and used for footprint assays as described in a. No *Kr* sites are observed, but the fragment contains at least four Hb sites. One of them Hb18, contains four copies of the 10-bp consensus sequence. Lanes 2 (—) and 5 (C) in both autoradiograms are controls, as described in Fig. 2 legend. Lane 1 (GA) in each autoradiogram corresponds to the deoxyadenosine and deoxyguanosine sequences of the DNA fragment.

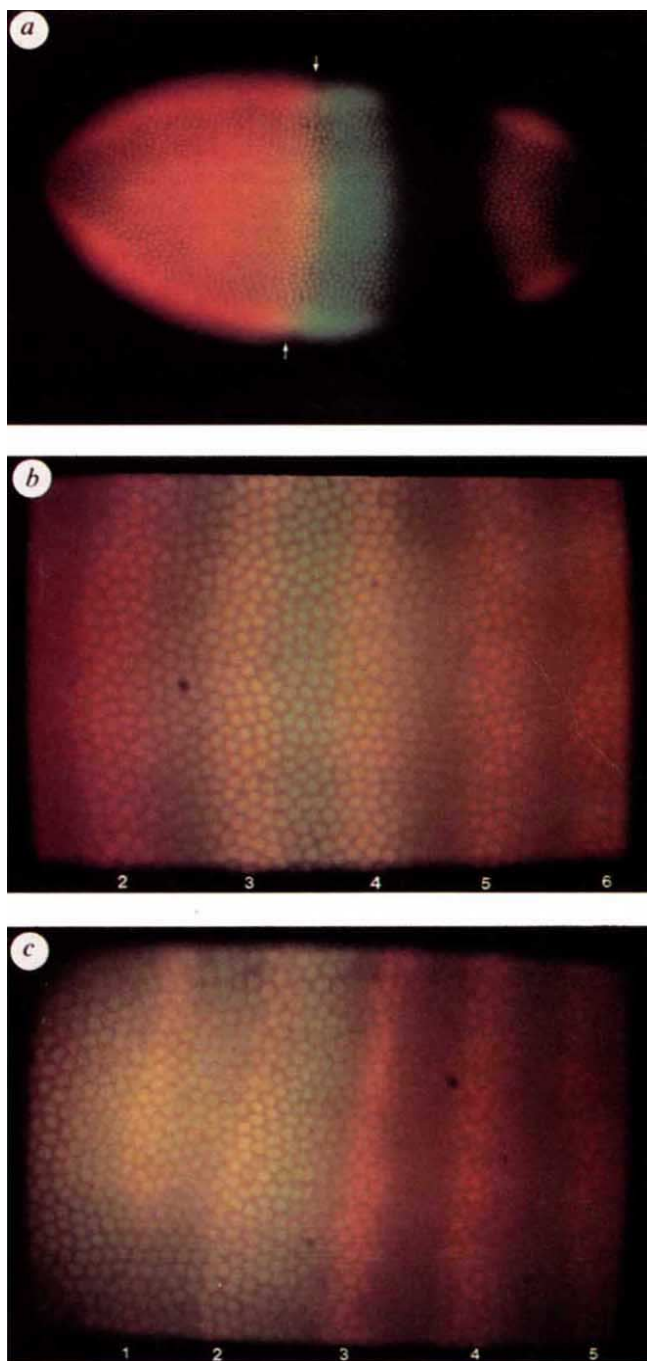


FIG. 4 Co-localization of *hb*, *Kr* and *eve* proteins in wild-type embryos. *a*, Embryo undergoing cellularization stained with a mixture of antibodies against *hb* proteins (red) and against *Kr* proteins (green). Cells that express both proteins stain yellow and are indicated by arrows. *b*, High magnification view of an embryo stained with a mixture of antibodies against *eve* proteins (red) and *Kr* proteins (green). The numbers at the bottom correspond to *eve* expression stripes 2–6. The distribution of the *Kr* protein shows a broad, bell-shaped profile, extending from the posterior margin of *eve* stripe 2 through to the anterior margin of stripe 5. *c*, High magnification view of an embryo stained with a mixture of antibodies against *eve* proteins (red) and *hb* proteins (green). Only the anterior domain of *hb* expression is observed. The protein shows a graded distribution, with more posterior regions expressing lower levels of *hb*. Expression encompasses stripes 1 and 2, and low levels of *hb* protein extend into cells expressing *eve* stripe 3. Note that stripe 3 is brighter than stripes 4 and 5, due to the appearance of both *hb* and *eve* proteins.

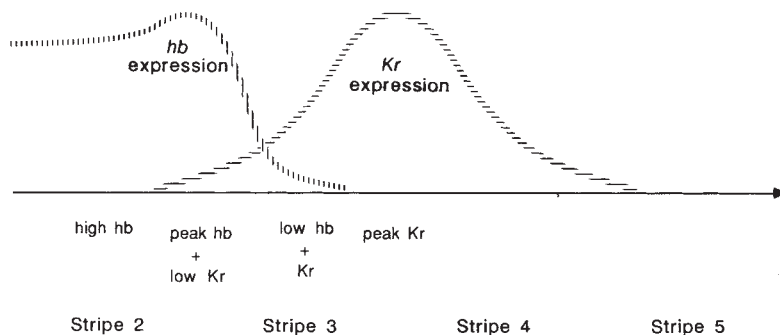
METHODS. Embryos were fixed and stained with antibodies as described⁸. Antibodies were: rat anti-*hb* protein, rabbit anti-*Kr* protein, and guinea-pig anti-*eve* protein. Whole sera were diluted in PBS buffer (rat, 1:200; rabbit, 1:500; guinea pig, 1:100) and preabsorbed overnight at 4 °C with fixed embryos of mixed stages. Indirect immunofluorescence was done with TRITC-conjugated anti-rat and anti-guinea pig secondary antibodies, and with an FITC-conjugated anti-rabbit antibody (Jackson Immuno Research). Photomicrographs were taken with a Nikon Optiphot fluorescence microscope. Double exposures were done by separately photographing with the fluorescein and rhodamine channels. Kodachrome daylight film (ASA 64) was used to obtain transparencies, which were printed on Cibachrome paper (Ilford).

cells that comprise stripe 3 (Fig. 4c). The patterns of *hb*, *Kr* and *eve* expression are summarized in Fig. 5.

Our results show that the *hb* and *Kr* proteins possess sequence-specific DNA-binding activities. Previous studies indicated such a role for these proteins, but failed to provide definitive proof^{12,13,20–24}. The *Kr* protein binds with a high degree of specificity because each of the *Kr* sites contains a well-conserved copy of a 10-bp consensus sequence (see Fig. 1). The *Kr1* site contains a 10/10 match with the consensus, and showed the highest relative affinity for the protein (data not shown). The *hb* protein seems to bind DNA cooperatively because the highest affinity sites were found to contain multiple copies of the consensus sequence of the *Hb* sites. Binding sites that include single copies of the consensus showed a lower affinity for the protein (data not shown).

It is probable that at least some of the *Hb* and *Kr* sites that we identified in this study play a part in mediating the complex regulatory interactions between gap genes and the *eve* promoter defined in previous genetic studies^{8,17}. Goto *et al.*¹⁷ found that a 600-bp region of the *eve* promoter (from –1.7 to –1.1 kilobases (kb)) is particularly important for the expression of stripe 2. We have shown that this region contains one *Hb* site (*Hb3*) and four *Kr* sites (*Kr3*–*Kr6*; see Fig. 1); a 480-bp deletion that removes these sites (except *Kr6*) abolishes the expression of

FIG. 5 *hb*, *Kr* and *eve* expression patterns. The *hb* and *Kr* proteins are expressed in broad, overlapping gradients, such that a transverse stripe of about eight cells in width expresses significant levels of both proteins. The *eve* stripes 2–5 are shown. Each *eve* stripe includes a swath of about four cells, and interstripe regions also encompass four cells. This diagram is not intended to represent the distribution of *hb* and *Kr* products in a quantitative manner, but instead simply summarizes the approximate extent of overlap among the three patterns. The *eve* stripes 2 and 3 are expressed in regions of the early embryo where there are distinctive combinations and concentrations of the *hb* and *Kr* proteins. Stripe 2 coincides with cells expressing high levels of *hb* protein, but lacking *Kr*. Stripe 3 coincides with cells expressing low levels of *hb* protein and high levels of *Kr* protein. The inter-stripe region separating stripes 2 and 3 contain peak levels of *hb* protein and low levels of *Kr*. The 'bulge' in *hb* protein expression at this site (parasegment 4) is due to the superimposition of *hb* products specified by both the proximal and distal *hb* promoters (after fertilization, the distal



promoter is active only in parasegment 4)³⁰. The periodic on/off pattern of *eve* expression could involve these different combinations and concentrations of the *hb* and *Kr* proteins.

stripe 2 (ref. 17). Similarly, removal of a 900-bp region of the stripe-3 element (from -3.8 to -2.9 kb) that contains 11 Hb sites (Hb4-Hb14) eliminates the expression of stripe 3¹⁷. Furthermore, we have sequenced a total of 6.4 kb of the *eve* 5' flanking region (T.H. and M.L. unpublished results), and with very few exceptions the only sequences that share significant homologies with the consensus of the Hb and Kr sites are contained within the limits of the stripe-2- and stripe-3 elements shown in Fig. 1. DNA-binding studies support this correspondence between the occurrence of Hb- and Kr-site recognition sequences and functional regions of the *eve* promoter. For example, the most proximal 400 bp of the *eve* promoter is not essential for initiating stripes 2, 3 or 7 (refs 16, 17) and DNA-binding assays have failed to identify any *hb* or *Kr* protein binding activity in this interval (data not shown). Similarly, there do not seem to be any high-affinity binding sites in regions that map upstream of the stripe-3 element, including the distal autoregulatory element.

Perhaps the on/off periodicity of the *eve* promoter depends on distinctive concentrations and combinations of the *hb* and *Kr* proteins (for a summary see Fig. 5). Stripe 2 coincides with cells expressing high levels of *hb* protein and no *Kr* protein, whereas stripe 3 is expressed where there are low levels of *hb* protein and high levels of *Kr* protein. Genetic studies indicate that *hb* and *Kr* differentially regulate *eve* expression⁸. The *hb* gene activity exerts a positive effect on the expression of *eve* stripes 2 and 3, whereas *Kr* activity represses the expression of stripe 2. In *Kr*⁻ mutant embryos, the posterior margin of stripe 2 expands posteriorly into cells that normally contain appreciable levels of the *Kr* protein (ref. 8; R. Warrior & M.L., unpublished results; see Fig. 5). Perhaps the *hb* protein activates the expression of stripe 2 by binding to one or more of the sites in the stripe-2 element (see Fig. 1). Cells just posterior to the limit of stripe 2 seem to contain sufficient levels of the *Kr* protein, above a minimal threshold, to block activation by *hb*. Given the close linkage between the Hb- and Kr sites in the stripe-2 element, it is conceivable that the *Kr* protein blocks activation mediated by the *hb* protein by binding competitively to DNA. Alternatively, the *Kr* protein may mask (or quench) the activity of the *hb* protein through protein-protein interactions. We favour this latter mechanism, because the two proteins can occupy closely linked Hb and Kr sites; in fact it is possible that the two proteins bind to these sites cooperatively (Fig. 2; compare lane 9 with lanes 2 and 5). Similar models of protein-protein interactions have been proposed to account for the repression of α -specific genes in yeast by a complex of the cell-type-specific repressor $\alpha 2$ and the non-cell-type-specific protein PRTF²⁵, and the repression of heterologous promoters that contain multiple recognition sites for homeodomain proteins in transient co-transfection assays²⁶. □

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The products of the *Drosophila* gap genes *hunchback* and *Krüppel* bind to the *hunchback* promoters

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THE first zygotic genes to be expressed during early *Drosophila* development are the gap genes¹⁻³. Their role is to read and interpret coarse positional information deposited in the egg by the mother⁴⁻⁶ and to refine it by cross-regulatory interactions⁷ and by controlling a class of pair-rule genes^{8,9}. Little is known about the molecular mechanisms¹⁰ by which the three cloned gap genes^{3,11,12} carry out their genetically defined functions. Here we report that the *Krüppel* (*Kr*) gene product (*Kr*) binds to the sequence AAGGGGTAA, whereas the *hunchback* (*hb*) gene product (*Hb*) recognizes the consensus ACNCAAAAAAATA. We have identified binding sites for these proteins upstream of the two *hb* promoters, which we suggest could mediate the repression of *hb* by *Kr*⁷ and perhaps allow *hb* to influence its own expression.

The presence of zinc-finger motifs¹³ in both the *Kr* and *Hb* proteins^{3,11} indicates that these proteins can bind to DNA. To identify fragments that contained binding sites for *Kr* and *Hb*, we used an immunoprecipitation assay based on our observation that *Kr* and *Hb* overproduced in *Escherichia coli* are insoluble but still able to bind to DNA. Such binding sites were searched for in a complex DNA mixture (the 50-kilobase (kb) bacteriophage lambda genome), which should by chance have contained sequences approaching those actually recognized by the *Kr* and *Hb* proteins *in vivo*¹⁴⁻¹⁶. We then used the protein extracts to footprint the binding sites on the precipitated fragments. Figure 1a presents four sequences protected by *Kr* and the consensus AAGGGGTAA derived from them. A synthetic DNA fragment that contained three repeats of this sequence exhibited strong footprints with the *Kr* extract, but none with the *Hb* extract (data not shown).

We then looked for biologically relevant *Kr*-binding sites in the regions flanking the two *hb* promoters³. The proximal promoter directs early zygotic expression in the anterior part of the embryo and is dependent on the maternal gene *bicoid*¹⁷⁻²⁰. In the absence of *Kr*, *hb* expression expands posteriorly into the *Kr*-expression domain, indicating that *Kr* normally functions to repress *hb* transcription⁷. The distal *hb* promoter is transcribed maternally^{3,17} and also directs later zygotic expression in one stripe at the border between *Kr* and early *hb* expression, and in another stripe near the posterior end of the embryo¹⁷.

The short time span of the interactions between gap genes implies that *Kr* acts directly on *hb*. We therefore searched for *Kr*-binding sites on a 10-kb genomic DNA fragment that contained the *hb* coding sequence and both *hb* promoters³. We identified two binding sites located at -676 and -359 base pairs (bp) from the proximal *hb* promoter (Fig. 1b); the footprint of one site is shown in Fig. 2. The sequences of the sites closely

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Kr-binding sites		Hb-binding sites	
In Lambda:		Upstream of the <i>hb</i> proximal promoter:	
2131	ACGGGGTTTA	-178 (Hb1)	AGAAACAAAAATTAT
2299	AAGCGTTAT	-659 (Hb2)	AAACGCAAAACGGGAA
2306	AAGGGGATGA	-2533 (Hb3)	ATAATTAACAACATAT
36483	GACGGGTAA		
Consensus		Upstream of the <i>hb</i> distal promoter:	
Score (/4)	3333443423	-449 (Hb4)	GATGCCAAAAACGGC
		-535 (Hb5)	TTGCCAAAAACAAT
		-731 (Hb6)	TAGCTTAAAAAGGTGG
		-831 (Hb7)	TTGGGAAAAAATTGG
		-915 (Hb8)	GGACTAAAAAAAATA
		Consensus	ACNCAAAAAANTA
		Score (/8)	44 4888776 45
Upstream of the <i>hb</i> proximal promoter:			
-359 (Kr1)	TGCGGGACTTAA		
-676 (Kr2)	AAGGGGCATTTA		

FIG. 1 *a*, Sequences recognized by the Kr and Hb proteins. Four sites in lambda DNA protected from DNase I were used to define the Kr-binding consensus sequence; Kr-binding sites upstream of the proximal *hb* promoter are also indicated. Eight sites, which are upstream of both *hb* promoters, have been footprinted by the Hb-protein extract. The protected sequences are shown together with the consensus derived from them. *b*, Summary of the binding sites detected for Kr (short vertical arrows) and Hb (long vertical arrows, above the line) in the regions of the *hb* promoters^{3,17}. The positions of *bicoid*-gene-product (Bcd) binding sites, according to Driever and Nüsslein-Volhard¹⁸ (long vertical arrows, below the line) are also shown.

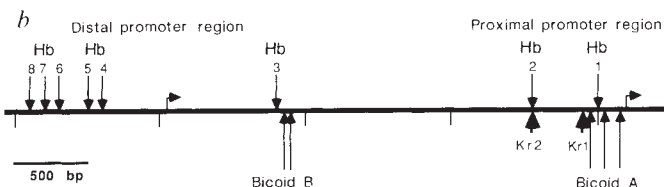


FIG. 2 DNase I footprints of binding sites for Kr and Hb flanking the *hb* promoters. G/A, Maxam and Gilbert sequencing ladder. *a*, -298 to -802-bp region of the proximal *hb* promoter, showing a Kr-binding site at -359 bp (Kr1). lanes 0, no protein extract. The other lanes contained an amount of protein extract from bacteria (containing the control pAR3040 (C lanes) or the pARKr (Kr lanes) expression plasmids) as follows (μ g): lane 1, 0.2; lane 2, 1.0; lane 3, 5.0; lane 4, 25.0. *b*, -667 to -387-bp region of the distal *hb* promoter, showing Hb-binding sites at -449 bp (Hb4) and at -535 bp (Hb5). *c*, -667 to -1019-bp region of the distal *hb* promoter, showing Hb-binding sites at -731 bp (Hb6), -831 bp (Hb7) and -915 bp (Hb8). In *b* and *c* lanes 0 contained no extract. The other lanes contained an amount of bacterial extract (containing the pAR3040 (C lanes) or pARHb (Hb lanes) expression plasmids) as follows (μ g): lane 1, 0.2; lane 2, 1.0; lane 3, 5.0; lane 4, 25.0.

METHODS. The pARHb plasmid was constructed by creating *Nde*I and *Bam*HI sites at the ends of a 2.4-kb genomic *Xba*I fragment^{3,18} that contained the entire *hb* coding sequence³, using amplification by the polymerase chain reaction with oligonucleotide primers that contained these sites. The resulting *Nde*I-*Bam*HI fragment was cloned into pAR3040, a T7 expression vector²⁴. *E. coli* BL21 (DE3) that contained either pARKr (provided by Hoey and Levine), pARHb or pAR3040 were induced with 0.4 mM IPTG at $A_{600} \sim 0.5$, and extracts were prepared by denaturation in buffer Z containing 4M guanidine-HCl, followed by renaturation against buffer Z, according to the method of Hoey and Levine²⁵, except that the buffer contained no $MgCl_2$ and did contain 0.5 mM $ZnCl_2$ and 0.2 mM EDTA. The presence of zinc was not essential but did seem to result in higher activity. All labelled fragments

were derived from pE8B1000, a 10-kb *Bam*HI genomic *hb* clone³ (provided by Gaul and Jäckle). The fragment in *a* was labelled at the *Sal*I site and recut with *Hind*III; *b*, Fragment was labelled at the *Hind*III site and recut with *Hha*I; *c*, Fragment was labelled at the *Hind*III site and recut with *Bam*HI. DNase footprint methods: 5×10^4 c.p.m.-labelled DNA was incubated for 30 min at 0 °C in 25 μ l binding buffer (20 mM Tris-HCl pH 7.5, 50 mM NaCl, 0.2 mM EDTA, 0.5 mM $ZnCl_2$, 800 μ g ml^{-1} poly(dI.dC), 10% glycerol). Then 175 μ l footprint dilution buffer (10 mM Tris-HCl pH 7.5, 1.2 mM $MgCl_2$, 0.25 mM $CaCl_2$, 1 mM DTT, 10 μ g ml^{-1} salmon sperm DNA, 10% glycerol) was added, followed by DNase I to a final concentration of 6.25 $ng\ ml^{-1}$. After 5 min at 0 °C the reaction was stopped with EDTA and NaCl at final concentrations of 10 mM and 300 mM, respectively, and the samples were purified by phenol-chloroform extraction and ethanol precipitation before electrophoresis on an 8% sequencing gel.

match the consensus, except that they both contain a 2-bp insertion in the middle of the most highly conserved region (Fig. 1a). Because Kr contains four zinc fingers, relative motion between them could allow for such flexibility in the spacing of these conserved base pairs.

We are now testing short-lived *hb-lacZ* gene fusions²¹ to determine whether the binding sites that we have described mediate repression of *hb* by *Kr*⁷.

By using the same approach, we identified binding sites for the Hb protein that are upstream of both promoters of the *hb* gene (Fig. 1b). Figure 2 shows examples of DNase I footprints obtained with a crude extract that contained the Hb protein, on fragments initially identified by the precipitation assay. The

consensus sequence for Hb binding is ACNCAAAAAAANTA (Fig. 1a), which is quite distinct from the consensus bound by Kr. The central run of adenines is the most highly conserved part of the Hb-binding consensus. Repeats of the synthetic consensus sequence ACTCAAAAAACTA were protected from DNase I by Hb, but not by Kr (data not shown).

The presence of Hb-binding sites upstream of the *hb* promoters indicates that this gene could be autoregulated, as are some other developmental genes²¹⁻²³. We are now testing the functions of these sites *in vivo*.

In conclusion, we have identified specific binding sites for the Kr and Hb proteins upstream of the *hb* promoters, through which Kr and Hb could regulate expression of *hb*. □

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Krüppel requirement for *knirps* enhancement reflects overlapping gap gene activities in the *Drosophila* embryo

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SEGMENTAL pattern formation in *Drosophila* proceeds in a hierarchical manner whereby the embryo is stepwise divided into progressively finer regions until it reaches its final metameric form^{1,2}. Maternal genes initiate this process by imparting on the egg a distinct antero-posterior polarity and by directing from the two polar centres the activities of the zygotic genes³. The anterior system is strictly dependent on the product of the maternal gene *bicoid* (*bcd*), without which all pattern elements in the anterior region of the embryo fail to develop⁴. The posterior system seems to lack such a morphogen. Rather, the known posterior maternal determinants simply define the boundaries within which abdominal segmentation can occur, and the process that actively generates the abdominal body pattern may be entirely due to the interactions between the zygotic genes⁵⁻⁷. The most likely candidates among the zygotic genes that could fulfil the role of initiating the posterior pattern-forming process are the gap genes, as they are the first segmentation genes to be expressed in the embryo⁸⁻¹⁰. Here we describe the interactions between the gap genes *Krüppel* (*Kr*), *knirps* (*kni*) and *tailless* (*tll*). We show that *kni* expression is repressed by *tll* activity, whereas it is directly enhanced by *Kr* activity. Thus, *Kr* activity is present throughout the domain of *kni* expression and forms a long-range protein gradient, which in combination with *kni* activity is required for abdominal segmentation of the embryo.

Mutations in the gap genes *Kr*, *kni* and *tll* cause large pattern-

deletions in the abdomen: *Kr* affects the development of thoracic (T) and abdominal (A) segments T1 to A6 (ref. 11), *kni* affects abdominal segments A1 to A7 (ref. 12), and *tll* affects segments posterior to A7 (ref. 13). The gene *kni* is expressed in three areas at the cellular blastoderm stage¹⁴ (Fig. 1a, b). The broad posterior domain of expression (the lack of which is most probably responsible for the gap phenotype) narrows from the posterior border during early stages of development so that the initial domain of *kni* expression pattern covers ~23-45% egg length, whereas during cellulation it spans ~30-45% (the posterior pole is 0%; for details see ref. 14). First we investigated whether the neighbouring gap genes, *Kr* and *tll*, played any part in establishing the posterior pattern of *kni* expression.

In *tll*-mutant embryos, the domain of *kni* expression is expanded towards the posterior pole (Fig. 1c). The simplest explanation for this is that *tll* activity represses *kni* expression, and that the lack of *tll* activity in the mutants allows *kni* to be ectopically expressed in a region where *tll* would normally be active. An unexpected result was obtained in *Kr*-mutant embryos. We did not see the anticipated expansion of the *kni* expression domain towards the anterior pole, but instead we saw a marked decrease in the level of *kni* expression in its normal posterior domain relative to that in its anterior domain (Fig. 1d). These observations indicate that *Kr* activity enhances *kni* expression, whereas *tll* limits the posterior border of *kni* activity by acting as a repressor.

To identify the *cis* regulatory elements of *kni* that mediate its interactions with *tll* and *Kr*, we made fusion constructs between the upstream sequences of *kni* and the bacterial *lacZ* gene (Fig. 3a). The constructs were integrated into the *Drosophila* germ line using P-element mediated transformation¹⁵, and the expression pattern of β -galactosidase (β -gal) was monitored in early embryos. A construct containing ~4.4 kilobases (kb) of *kni* upstream sequence (4.4 *kni-lacZ*) gave the correct spatial pattern of expression in the anterior and the posterior domains (Fig. 2a, b). When this construct was placed into a *tll*-mutant background, the expression of β -gal in the posterior domain expanded towards the posterior pole, as had been observed for the endogenous *kni* expression (Fig. 2c). In addition, we crossed

the 4.4 *kni-lacZ* fusion gene construct into the background of a gain-of-function mutant for the *torso* gene^{16,17}. This maternal mutation eliminates all segments in the thoracic and abdominal region and the effect is thought to be due in part to the ectopic expression of *tlh* in the segmental primordia^{16,17}. As shown in Fig. 2e, the posterior expression of β -gal is absent in these mutant embryos, consistent with the view that *tlh* is a repressor of *kni* activity.

The 4.4 *kni-lacZ* fusion construct was then introduced into *Kr* mutants to see if the 4.4-kb fragment of *kni* also contained the *cis* regulatory elements that mediated *Kr*-dependent enhancement of *kni* expression. As shown in Fig. 2d, the level of β -gal expression in the posterior domain was reduced relative to the anterior domain, which served as an internal standard. This demonstrated that the *Kr*-responsive elements in *kni* resided in this 4.4-kb fragment.

We then investigated whether the *Kr*-gene product directly interacts with the *kni* upstream sequence. The 4.4-kb *kni* upstream DNA was screened for potential *Kr* protein-binding sites by immunoprecipitation¹⁸. Using a truncated form of bacterially produced *Kr* protein that contained the DNA-binding finger domain, we detected one DNA fragment that specifically precipitated with the *Kr* protein and antibody (Fig. 3b). DNase I footprinting¹⁹ was used to characterize further the binding sites within this fragment. Two adjacent *Kr* protein-binding regions contained the sequence AAAAGGGTTAA (Fig. 3c).

To assess the *in vivo* relevance of these *in vitro* binding sites, we deleted the region that contained the *Kr* protein-binding sites from the 4.4 *kni-lacZ* construct and reintroduced the modified fragment (3.6 *kni-lacZ*) into flies. As shown in Fig. 2f, this construct gave a decreased expression of β -gal in the posterior domain relative to the anterior domain. This indicated that the sequences characterized *in vitro* are biologically important sites for the binding of the *Kr* protein. Of course, we do not know whether sites that were not detected by the footprinting but included in the deleted region also contributed to the decrease in β -gal expression. Because a low level of *kni* expression still exists in *Kr* mutants, the initial spatially restricted expression of *kni* must be dependent on factors other than *Kr*. At the moment we do not know whether a basal level of *kni* expression must be present before *Kr* can enhance the expression of *kni*, or whether *Kr* can activate *kni* transcription *de novo*. In either case, the results that we present have indicated that *Kr* activity is present and required throughout the domain of *kni* expression, and that *Kr*-gene product sets the correct level of *kni* expression by acting as a sequence-specific transcription regulator.

A long-standing dilemma has been the difference in size between the domain of *Kr* expression in wild-type embryos and the region affected in the phenotype of *Kr* mutants²⁰. The pattern of *Kr* transcription revealed by *in situ* hybridization⁸, as well as the initially described *Kr* protein domain²¹, cover only about

FIG. 1 Localization of *kni* transcripts by whole-mount *in situ* hybridization. Expression of *kni* in wild-type- and mutant embryos. Orientation of embryos is anterior-left and dorsal-up. *a*, Wild-type embryo at syncytial blastoderm; *b*, Wild-type embryo at cellular blastoderm; *c*, *tlh*⁶ embryo at syncytial blastoderm; *d*, *Kr*¹ embryo at syncytial blastoderm. Note the anterior and posterior domains of *kni* expression in wild-type embryos (*a*, *b*), the expansion of the posterior domain towards the posterior in the *tlh* mutant (*c*), and the decrease in the posterior domain relative to the anterior in the *Kr* mutant (*d*). The *kni* complementary DNA probe pCJ15 (ref. 10) and the staining procedure of Tautz and Pfeifle³⁴ were used.

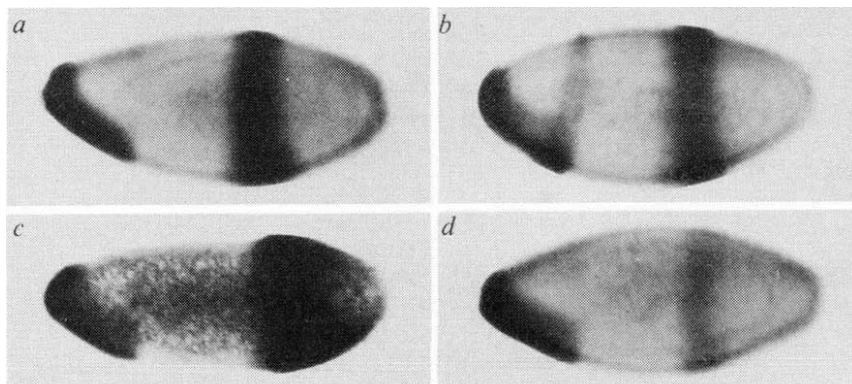
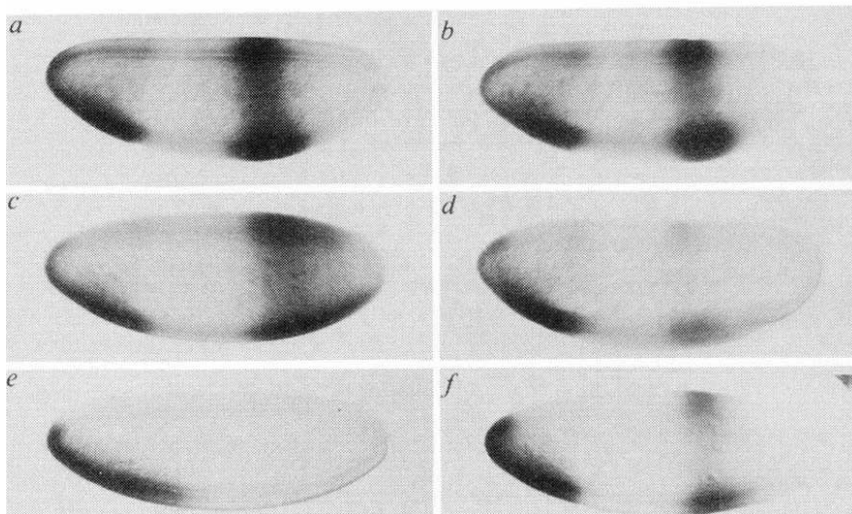


FIG. 2 Expression of β -galactosidase under the control of *kni* upstream sequences. Embryos in *a* to *e* harbour the 4.4-kb *kni-lacZ* construct (Fig. 3a). The embryo in *f* harbours the 3.6-kb *kni-lacZ* construct, which has the 800-base pair (bp) fragment containing the *Kr* protein-binding sites deleted (see Fig. 3). *a*, Wild-type embryo at late cellular blastoderm; *b*, Wild-type embryo at early gastrulation. Because of the stability of the RNA and/or protein, β -gal persists throughout development. Note that the relative levels of β -gal in the anterior and the posterior domains are about equal; *c*, *tlh*⁶ embryo. The posterior domain expands towards the posterior; *d*, *Kr*¹ embryo. The level of β -gal posteriorly is decreased relative to that anteriorly; *e*, *tor*⁴⁰²¹ gain-of-function mutant embryo. The posterior domain is absent; *f*, wild-type embryo carrying the 3.6 *kni-lacZ* construct in which the region containing the *in vitro* *Kr* protein-binding sites has been deleted (see Fig. 3). Note again that the level of β -gal posteriorly is decreased relative to that anteriorly. Orientation of the embryos is anterior-left and dorsal-up. Embryos in *b* and *d* are at about the same stage of development, as are the embryos in *a*, *c*, *e* and *f*. METHODS. The appropriate *kni* DNA fragments were cloned into the P-element vector pCaSpeR AUG β -gal (ref. 35), transformed into flies¹⁵, and



the embryos harbouring the constructs assayed for β -gal expression. For each experiment, at least two independent transformant lines were tested. All embryos were stained with antibodies against β -gal as previously described³⁶.

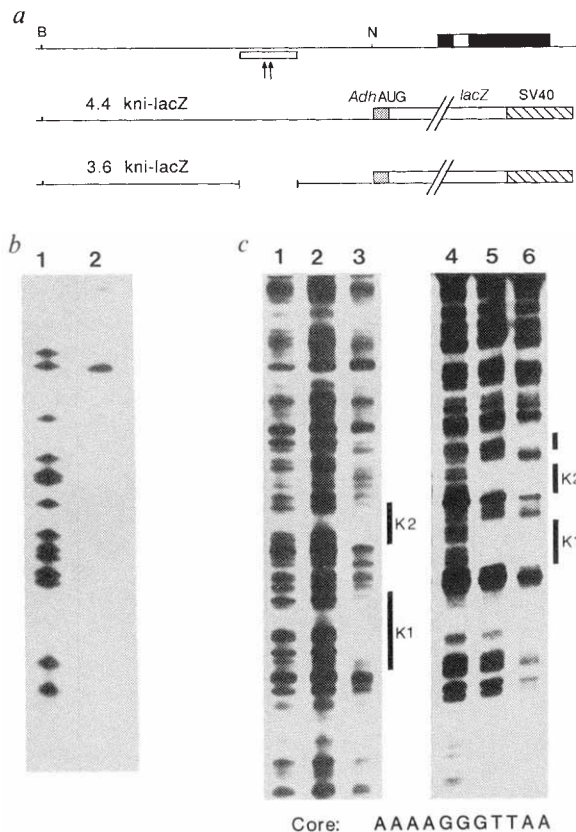


FIG. 3 Identification of *Kr*- and *tll* responsive elements in the *kni* upstream region. **a**, Top line shows the genomic map of *kni*. Black boxes are coding regions¹⁰. Middle line shows the fusion construct between 4.4-kb *Bam*H1(B)-*Nru*I(N) upstream fragment of *kni* and the pCaSpeR AUG β -gal³⁵. The bottom line shows *kni-lacZ* construct in which an 800-bp fragment containing the *Kr* protein-binding sites has been deleted. The unshaded rectangle below the top line indicates the fragment that specifically immunoprecipitates with *Kr* protein and antibody. The two arrows indicate the approximate positions of the sequence protected by *Kr* protein in the DNase I footprinting assay. **b**, Immunoprecipitation of *kni* upstream sequences. Lane 1 shows input DNA consisting of the 4.4-kb *kni* upstream fragment digested with *Hpa*II and end-labelled. Lane 2 shows the immunoprecipitated DNA fragment. One DNA fragment of ~800 bp specifically precipitates with extract containing *Kr* protein. No fragment precipitated with control extract made from bacteria that do not express *Kr* protein (not shown). Using a different restriction enzyme (*Taq*I), the fragment was narrowed down further to ~400 bp (not shown). This fragment was used for DNase I footprinting. **c**, DNase I protection of *kni* upstream sequence. Left panel shows the coding strand labelled at the 3' end. Lane 1, no protein extract. Lane 2, 1.2 μ g control extract without *Kr* protein. Lane 3, 1.2 μ g extract with *Kr* protein. Lane 4, no protein extract. Lane 5, 4 μ g *Kr* extract. Lane 6, 16 μ g *Kr* extract. The sequence protected in the coding strand is AAAAGGGTTAAACAGG for K1 and AAAAGGGTTAAG for K2. The sequence protected in the non-coding strand is AATCGCGGTATTTTCCCAATTT for K1 and TTACGCGCGGTATTTTCCCAATTC for K2. Therefore the common core sequence protected on both strands is AAAAGGGTTAA (reading from the coding strand). More sites become protected with increasing *Kr* protein concentration, as demonstrated by the region marked above the K2 site to the right of lane 6. **METHODS.** *Kr* protein was produced in bacteria using the T7 promoter system³⁷. *Kr* DNA fragment used in the expression vector started from the *Bam*H1 site located 5' to the region encoding the DNA-binding finger motifs³⁸. The extract was prepared as previously described³⁹. For immunoprecipitation, crude extract was used. For DNase I footprinting, the crude extract was passed over a heparin-agarose column and eluted with KCl. The fractions were tested for the presence of *Kr* protein by western analysis and subsequently pooled. The control extract was prepared in the same way from bacteria carrying the expression vector alone without any *Kr* sequences.

half the size of the blastoderm anlagen that are affected in the mutants. It now seems, however, that these observations were limited by the degree of visual resolution, because improved staining techniques have recently allowed the detection of a much broader domain of *Kr* expression²². Our observation that the product of *Kr* directly binds to, and enhances the transcription of, *kni* demonstrates that *Kr* activity is not limited to the central region of the embryo. Rather, the *Kr* protein forms a steep asymmetric gradient, which extends significantly into the posterior region. Further evidence for the long-range effect of *Kr* activity is that the expression patterns of stripes five and six of the pair-rule gene *even-skipped* (*eve*), which lie outside the central high-level *Kr* domain, are altered in *Kr* mutants²³. For the pair-rule gene *hairy* (*h*), we have identified specific DNA fragments that control the expression of individual stripes of the *h* gene. The control element responsible for the *h* stripe six contains binding sites for the *Kr* protein and requires *Kr* activity for its normal expression (M.J.P., unpublished observations). These results provide further support for the presence and requirement of *Kr* activity in the posterior region of the embryo.

If the posterior body pattern in *Drosophila* is in fact set up solely through zygotic input⁵⁻⁷, then the process that generates the abdominal body pattern may be initiated by the activities of *Kr* and *kni*. Formation of the anterior body pattern requires the concentration-dependent positional information provided by the maternal morphogen *bcd* (refs 24-27); the gap genes *hunchback* (*hb*) (refs 26-29), *Kr* (ref. 30) and other possible target genes²⁶ respond differently to the different *bcd* protein concentrations constituting the *bcd*-protein gradient along the longitudinal axis in the early embryo^{26,27}. We suggest that there could be a system analogous to that for *bcd*, but at the zygotic level, in which the *Kr*-product in combination with other gap-gene products determines positional values in the posterior segmental region. The *Kr* gene alone cannot perform this function, because in a situation where only *Kr* is expressed throughout the embryo in the absence of *hb* or *kni* expression, no segmentation is observed³⁰. Furthermore, owing to the extensive overlap of gap gene activities, the relative concentrations between a given combination of gap gene products could direct diverse pattern-forming events. This would explain how so few gap genes could control a wide range of pattern-forming processes. It has already been shown for *eve* (refs 31, 32) and *h* (ref. 33) that individual pair-rule stripes possess unique identities, which are controlled by separable regulatory elements. The generation of the initial pair-rule expression patterns could, therefore, be directed by distinct positional cues that are potentially established by the overlapping gradients of the gene products of *hb* and *Kr* (ref. 22), and *Kr* and *kni*. □

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The caudal gene product is a direct activator of *fushi tarazu* transcription during *Drosophila* embryogenesis

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A *DROSOPHILA* pair-rule segmentation gene, *fushi tarazu* (*ftz*), encodes a protein which is expressed in a characteristic seven-stripe pattern¹. The promoter sequences that are sufficient for generating this spatially restricted pattern of expression are located within 669 base pairs upstream of the transcription start site². Multiple transcriptional activators and repressors interact with this 'zebra-stripe' promoter unit to bring about the positional specificity of *ftz* transcription³. Here we report that the homoeodomain-containing protein encoded by *caudal* (*cad*) is one such regulator. The *cad* gene product can increase the level of *ftz* transcription in the posterior half of the embryo by interacting with multiple copies of a TTTATG consensus sequence located in the zebra-stripe unit. This result demonstrates one pathway by which the product of a maternally expressed segmentation gene, expressed in an antero-posterior concentration gradient, can directly regulate the expression of a pair-rule gene.

An analysis of sequential deletions of the zebra-stripe promoter unit demonstrated that sequences between –482 and –386 base pairs (bp) from the startpoint of transcription can direct the expression of *ftz-lacZ* gene-fusion constructs preferentially in the posterior parasegments of germ-line transformed embryos (Fig. 1). The expression of β -galactosidase was observed in a continuous band rather than in discrete stripes, because the constructs did not contain the repressor elements that would normally prevent transcription in the cells that comprised the interband regions³. Staining was consistently seen between the parasegments that would correspond to *ftz* stripes 4–7, with stripe 7 staining most strongly. Weak staining was also detected in most embryos in the parasegments just anterior to the region corresponding to stripe 4.

The posterior-specific expression of these *ftz*-promoter fusions is similar to the pattern of expression that is observed for the *cad* protein during early embryogenesis^{4,5}. Because posterior *ftz* expression is drastically reduced in *cad*[–] mutant embryos⁴, and because the *cad*-gene product contains a homoeodomain, which indicates that the protein has a DNA-binding capability⁶, we examined whether the *cad*-gene product could function as a direct regulator of *ftz* transcription. To determine if the *ftz*-promoter region responsible for posterior-specific expression contained a *cad*-protein DNA-recognition-element (CDRE), we performed DNA-binding experiments with

bacterially overproduced *cad* protein. We found that this promoter fragment contains two elements that are bound *in vitro* by *cad* protein (Fig. 2a). Both of these elements contain two copies of a motif with the consensus TTTATG; the distal element has an inverted repeat of the motif separated by 4 bp, whereas the proximal element has a direct repeat of the sequence separated by 2 bp (Fig. 3a). This *cad*-protein DNA-recognition sequence is different from that reported for other *Drosophila* homoeodomain proteins (see ref. 7 for a review), which is consistent with the diverged nature of the *cad*-protein homoeodomain⁶. Additionally, at least two homoeoproteins, which recognize a TCAATTAAAT homoeodomain-binding consensus, the *even skipped* and *engrailed* proteins, do not bind *in vitro* to the TTTATG sequence (C.R.D., unpublished observations). These observations support the idea that the CDRE is specific for the *cad* protein.

Both of the elements to which the *cad* protein binds are required for the posterior expression of *ftz-lacZ* fusion constructs in germ-line transformed embryos. Point mutations in which the first and fourth base pairs of the consensus hexamer were changed to G and to T, respectively, eliminated the binding of the *cad* protein *in vitro* (Fig. 2b,c and Fig. 3). Mutations in either the two distal consensus sequences or in the two proximal consensus sequences also eliminated the posterior pattern of expression. The expression of β -galactosidase in embryos that were transformed with either of these mutant fusion genes resembled that of embryos transformed with gene fusions that completely lacked both the distal and proximal elements (Fig. 1). In addition to the negative effects that the mutations had on transcription, point mutations in one of the repeats seemed to reduce the level of binding of the *cad* protein at the remaining binding site ~100 bp away (Fig. 2b,c). This indicates that some form of cooperative binding may occur with *cad* protein. It has been suggested that homoeoproteins recognizing the TCAATTAAAT consensus sequence also bind cooperatively^{8,9}.

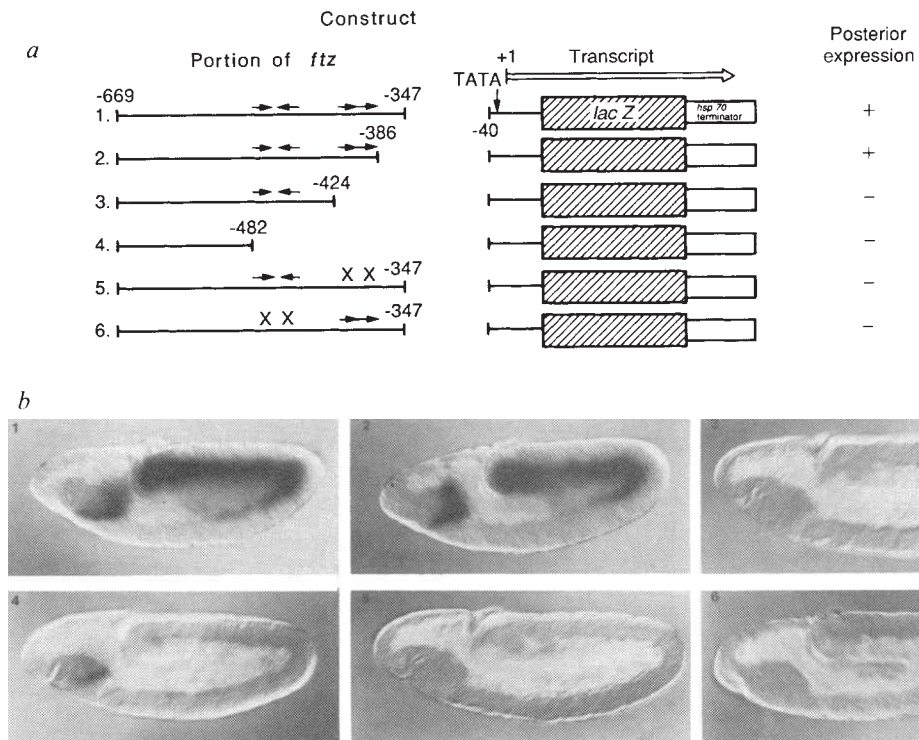
To demonstrate that *cad* protein can increase the transcription of the *ftz* gene by acting through the CDRE, we performed transient co-transfection assays in *Drosophila* Schneider-2 cells¹⁰. When *cad* protein was over-expressed in these cells, the increase in the transcription of a fusion construct that contained six copies of the TTTATG sequence was 12-fold more than the increase in the transcription of a construct containing six copies of a mutant hexamer (Fig. 4a). Furthermore, the transcription of intact *ftz*-promoter sequences which contained the CDREs was increased more than six times over that of a similar construct from which the binding sites had been deleted, and point mutations in either of the CDRE repeats reduced this transcriptional increase by 42–48% (Fig. 4b). This effect of CDRE point mutations on transcription differs from that found in germ-line transformants, in which mutations in either of the consensus repeat sites eliminated all detectable posterior-specific expression of the fusion constructs. This difference is probably due to the nature of the transient expression assay, in which artificially high levels of *cad* protein were present within the cell.

We suggest that *cad* protein activates *ftz* transcription in both ectodermal and mesodermal cells, even though primarily mesodermal *ftz-lacZ* expression was observed in the embryos reported here. The –6.1 to –3.4 kb upstream promoter region is necessary for full *ftz* expression in the ectoderm^{2,3,11}; it is possible that these sequences are also necessary for high levels of ectodermal *cad*-driven transcription. It is also possible that *cad* protein activates *ftz-lacZ* expression in low but relatively similar amounts in both ectoderm and mesoderm, and a mesodermal enhancer element within the wild-type *rosy* gene of the Carnegie 20 injection vector may increase the mesodermal expression to readily detectable levels¹².

The work reported here demonstrates that the *ftz* promoter is activated by a regulatory factor expressed with positional specificity in the embryo, and illustrates the usefulness of

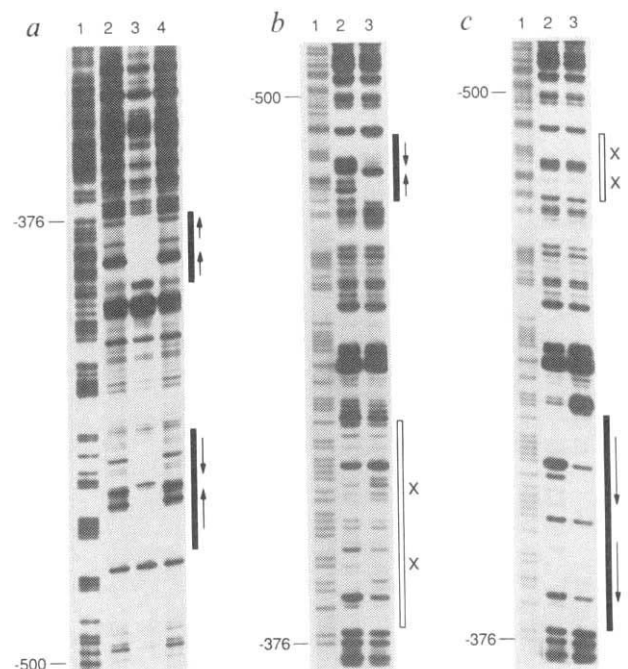
FIG. 1 Embryonic expression of germ-line-transformed *ftz-lacZ* gene-fusion constructs.

a, Diagram of the *ftz-lacZ* portion of the constructs injected and a summary of β -galactosidase expression observed in the germ band of transformed embryos. Numbers given are base pairs from the start point of transcription. Each arrow represents a single copy of the CDRE, whereas each X represents a mutated CDRE to which *cad* protein does not bind. **b**, Representative transformed embryo corresponding to the constructs summarized in **a**, stained for β -galactosidase activity using 5-bromo-4-chloro-3-indolyl-D-galactoside (X-gal) as substrate. Because these constructs lack the *ftz* upstream enhancer element and most of the zebra-stripe element, the expression levels are greatly reduced³. Therefore, embryos were examined during germ-band extension, as this is the earliest stage in which β -galactosidase activity (or antigen) can be readily detected. Constructs 1 and 2, which contained two intact copies of the CDRE repeats, expressed β -galactosidase in a posterior-specific pattern (see text). Constructs 3–6, in which one or both of the CDRE repeats were deleted or mutated, did not express β -galactosidase in the germ band. The cephalic staining observed is variable and is most probably due to elements in the Carnegie 20 injection vector used in these studies^{2,3,12}. **METHODS**. Promoter fragments of *ftz* were ligated to the 5 Δ -40 construct³, in which *ftz* sequences from -40 to +73 bp from the 5' cap site are fused to the *Escherichia coli lacZ* coding sequences. The construction of *ftz-lacZ*-fusion genes, generation of germ-line transformants, X-gal staining of embryos, and photography were as described previously³. Point mutations



(see Fig. 3 for base-pair alterations) were made with the Amersham oligonucleotide-directed *in vitro* mutagenesis system (version 2) according to manufacturer's instructions. At least five independent transformed lines were examined for each construct described. Embryos stained for longer periods of time than those presented show slight amounts of β -galactosidase activity in the germ band, apparently due to the presence of a general activator element located between -482 and -535 bp³. Constructs 1 and 4 were previously described in ref. 3 as 3' Δ -347 and 3' Δ -482, respectively.

FIG. 2 DNase I protection of the *ftz* promoter by *cad* protein. Numbers given are base pairs from the startpoint of transcription. Sequences protected from DNase I digestion are indicated by solid rectangles; sequences not protected because of the generation of point mutations are indicated by open rectangles. The approximate locations of the TTTATG consensus motif are shown with arrows; locations of the mutated consensus are shown with an X. **a**, Intact fragment of the *ftz* zebra stripe promoter unit. Lane 1, T and C nucleotides; lane 2, no extract added; lane 3, extract from bacteria expressing *cad* added; lane 4, crude extract from bacteria not expressing *cad* added. Two DNase I footprint regions are seen, specific for digestion reactions in which the *cad* extract had been added. **b**, Zebra-stripe promoter fragment of *ftz* containing mutated consensus binding sites at the proximal protection region. Lane 1, T and C nucleotides; lane 2, no extract added; lane 3, *cad* extract added. Two DNase I footprint regions are seen, specific for digestion reactions in which the *cad* extract had been added. **c**, Zebra-stripe promoter fragment of *ftz* containing mutated consensus binding sites at the distal protection region. Lane 1, T and C nucleotides; lane 2, no extract added; lane 3, *cad* extract added. Point mutations in the TTTATG consensus motif eliminated protection from DNase I digestion around those sequences, while reducing the level of protection at the intact footprinting region. **METHODS**. The *cad* protein was over-expressed in BL21 (DE3) bacteria using the system of Studier and Moffatt¹⁹. A T7/*cad* vector, p*cad*316, was used to overproduce *cad* protein. Induction of *cad* protein and production of crude extract were performed as described by Hoey *et al.*²⁰, with the exception that only the supernatant from the lysate centrifugation was used for all further steps. Crude *cad*-protein extract gave reasonably strong DNase I footprints (data not shown), but to optimize footprinting activity, *cad*-protein extract was subjected to chromatography on a heparin-agarose column. The flow-through was collected and the column washed in Z buffer (100 mM KCl, 25 mM HEPES buffer pH 7.8, 12.5 mM MgCl₂, 1 mM DTT, 0.1% Nonidet P-40 (NP-40), 20% glycerol, and protease inhibitors phenylmethylsulphonyl fluoride (1 mM) and benzamide (2 mM)). Fractions were then step-eluted by the sequential addition of Z buffer with 200 mM NaCl, 400 mM NaCl, and finally 700 mM NaCl. The majority of *cad* protein eluted in the 400 mM NaCl fractions, as determined by anti-*cad*-protein antibodies; these fractions were pooled, dialysed against Z buffer, and frozen at -70 °C until use. Heparin agarose eluate (15 μ l) was used for each footprinting reaction when required. Crude extract from bacteria not expressing *cad* protein was made from DE3 cells not transformed with p*cad*316, but otherwise treated in the same manner. This material (15 μ l) was used for each footprinting reaction when required. DNase I digestions were performed as described previously²¹, using ³²P-end-labelled DNA. Each reaction mix was electrophoresed on a 6% polyacrylamide gel, and the DNA fragments were detected by autoradiography.



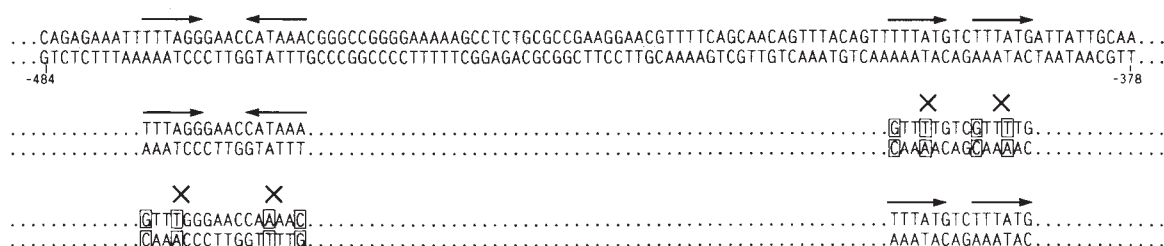


FIG. 3 Sequence of the *ftz* zebra-stripe promoter unit containing the *cad*-protein-recognition sites. *a*, Portion of the intact *ftz* promoter. Two repeats of the consensus motif are located between -474 and -388 bp from the startpoint of transcription. Three of the CDREs contain a TTTATG hexanucleo-

tide, whereas the fourth CDRE has a TTTAGG sequence. Each copy of the motif is indicated by an arrow. *b, c*, Portion of the *ftz* promoter in which point mutations have been introduced into the proximal CDREs (*b*) or into the distal CDREs (*c*). The mutant base pairs are boxed.

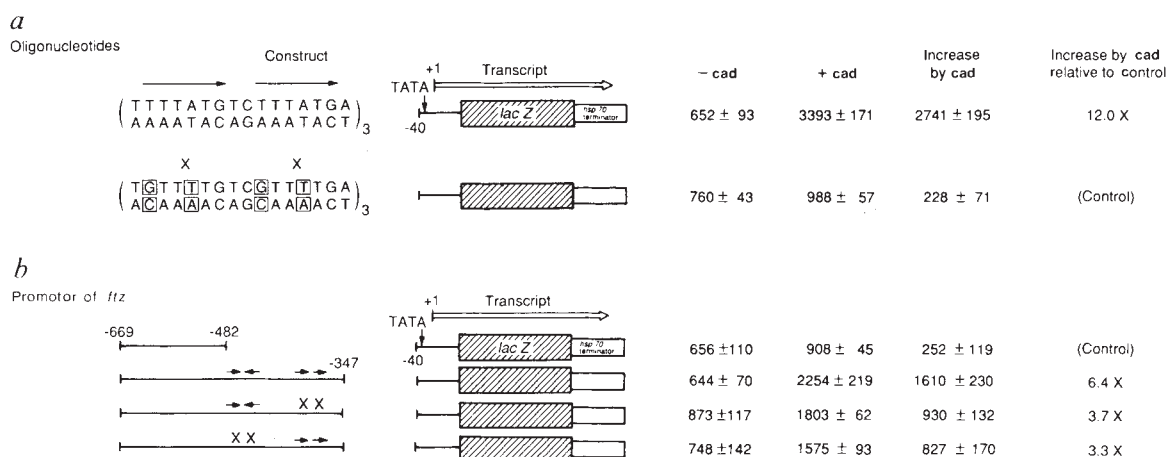


FIG. 4 Expression of CDRE fusion constructs in Schneider 2 cells overproducing *cad* protein. Fusion constructs were co-transfected with either the plasmid pP_{Ac} (ref. 10) (indicated as -cad) or the *cad* expression plasmid pP_{Ac}cad325 (indicated as +cad). The level of β -galactosidase activity in the cells was determined spectrophotometrically, and is given as arbitrary units. For each fusion construct, the increase in β -galactosidase expression due to overexpression of *cad* was determined by subtracting the activity in cells co-transfected with pP_{Ac} from that in the cells co-transfected with pP_{Ac}cad325. The calculated increases in expression levels were then compared, as indicated. *a*, Effects of *cad* over-expression on β -galactosidase activity of a fusion construct containing three copies of the proximal CDRE repeat. The control construct contains three copies of a CDRE repeat to which *cad* protein does not bind. Mutant base pairs are boxed. *b* Effects of *cad* over-expression on expression of constructs containing intact or mutated *ftz*-promoter fragments. Each intact CDRE repeat is indicated by

arrows, each mutant CDRE by an X. The control construct does not contain a CDRE repeat.

METHODS. The *cad* expression vector pP_{Ac}cad325 contains the *cad* coding region and the alcohol dehydrogenase gene polyadenylation signal, inserted into pP_{Ac}, which contains the actin 5C promoter. Fusion constructs, cloned into the Carnegie 20 vector, were co-transfected with either pP_{Ac} or pP_{Ac}cad325 according to the method of Krasnow *et al.*¹⁰, with the following alterations: 10 μ g of each plasmid and 5 ml of Schneider-2 cells were used for each individual experiment. After transfection, cells were grown for 48 h at 24 °C, collected, resuspended in 270 μ l assay buffer (50 mM potassium phosphate, 1 mM MgCl₂, pH 8.0) and frozen at -70 °C until further use. β -Galactosidase enzyme activity was determined spectrophotometrically by the method of Simon and Lis²², using the substrate chlorophenol red- β -D-galactopyranoside. Three independent experiments were performed and the results averaged for each value obtained.

detailed promoter dissections for elucidating the complicated regulation of *Drosophila* segmentation genes; indeed, the direct interaction between the *cad* protein and the *ftz* zebra-stripe promoter unit would have been difficult to predict from a comparison of the overall expression pattern of the *cad* and *ftz* proteins. It should be noted that other more general transcription factors also seem to contribute to activating *ftz* transcription through the zebra-stripe unit, even in the posterior half of the embryo (ref. 3, and our unpublished observations). Therefore it is possible that the extreme changes in *ftz* expression in *cad*⁻ mutant embryos⁴ are due to both direct and indirect effects of the mutation.

It has been postulated that the gap genes may act as intermediates between maternal and pair-rule segmentation genes¹³. Indeed, the maternally expressed *bicoid*-gene product can activate transcription of the gap gene *hunchback*^{14,15}. Furthermore, *cis*-acting elements that allow transcription in only one or a pair

of stripes, and which may respond to gap gene products, have been reported for the pair-rule loci *hairy* and *even-skipped*¹⁶⁻¹⁸. By contrast, the evidence so far indicates only an indirect regulation of *ftz* expression by the products of the gap genes; in addition, the data presented here demonstrate that the product of a maternally expressed gene, present in a concentration gradient along the antero-posterior axis, can interact directly with a pair-rule segmentation gene. Therefore, it seems that several types of hierarchical networks are used in the regulation of pair-rule gene transcription. □

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A nuclear DNA attachment element mediates elevated and position-independent gene activity

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THE transcriptional activity of genes that have randomly integrated into the genomes of transfected cells and transgenic organisms is in general unpredictable, varying with the chromosomal site of the insertion¹⁻⁴. This effect of chromosomal position on gene expression may reflect the organization of chromosomes into topologically constrained loops⁵⁻⁸ and functional domains⁹⁻¹². To assess the biological significance of these loop domains, the anchorage of DNA to the nuclear scaffold has been studied at specific gene loci^{8,13-20}. We have previously defined *cis*-acting regions flanking the chicken lysozyme-gene domain that mediate the attachment of the chromatin to the nuclear scaffold. These 'A-elements' map to the 5' and 3' boundaries of the region of general DNase sensitivity in the active chromatin^{10,17,21,22}, which contains the lysozyme gene and its *cis*-regulatory elements^{10,23-28}. Here we report that when a reporter gene is flanked by 5' A-elements from the lysozyme gene, its expression in stably transfected cells is significantly elevated and is independent of chromosomal position.

For the functional analysis of the A-element attachment-region, we constructed plasmids that each contained a 'mini-domain' consisting of one of various combinations of the enhancer of the lysozyme gene lying 6.1 kilobases (kb) upstream of the start site (E)²⁶, the lysozyme-gene promoter (P)²⁶, the reporter gene (C) encoding chloramphenicol acetyltransferase (CAT), and the lysozyme-gene 5' A-element (A), as depicted in Fig. 1. In the first series of experiments we transfected these plasmid DNAs into chicken HD11/HBCI-promacrophage cells, in which the endogenous lysozyme gene as well as the lysozyme enhancer are active^{17,26}, and measured the CAT activity 45 hours later (Fig. 1b). The results obtained with plasmids that contained

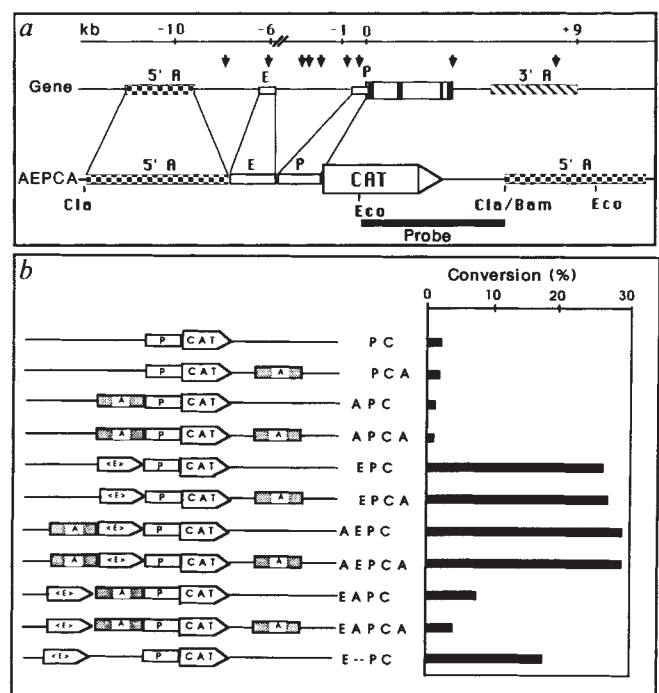


FIG. 1 Functional analysis of A-elements by their transient transfections into promacrophages. *a*, Diagram of the genomic organization of the lysozyme gene and of the construct containing an AEPCA mini-domain. In genomic location, the lysozyme domain (-12 kb to +9 kb) is flanked by 5' (dotted box) and 3' (hatched box) DNA attachment elements. The enhancer element²⁶ (nucleotides -6,331 to -5,772), the promoter element²⁶ (nucleotides -579 to +15) and the coding region with exons and introns (box with filled and open bars) are detailed. Arrowheads mark the positions of DNase-I-hypersensitive sites in the chromatin of various cell types^{23,25}. In the construct containing the AEPCA mini-domain (not to scale), the reporter gene *CAT*, which is linked to the lysozyme-gene promoter and enhancer, is flanked by two lysozyme-gene 5' A-elements (B1-X1 DNA fragment¹⁷, nucleotides -11.7 to -8.7 kb). Restriction sites relevant for Southern blot analysis (for details of probe, see legend to Fig. 2) are indicated: *ClaI* (Cla), *EcoRI* (Eco), *BamHI* (Bam). *b*, Constructs transfected into HD11/HBCI promacrophages^{17,26} and CAT activity in extracts of transiently transfected cells (44-48 h after transfection²⁶). Construction of plasmids containing PC (pLYSCAT2000) or EPC (pLYSCAT2100) or E-PC (pLYSCAT2300) was as described previously²⁶; plasmids containing the minidomains PCA, APC, APCA, EPCA, AEPC or AEPCA were constructed by blunt-end ligation of the lysozyme-gene 5' A-element into the *XbaI* and/or the *BamHI* site of plasmids containing PC or EPC. Plasmids containing EAPC or EAPCA were constructed by subcloning the enhancer fragment *BamHI*-*Sau3A*²⁶ into the *BamHI* site of the plasmid pB-1-X1¹⁷, reisolating a fragment containing the 5' A-element and the enhancer, and recloning by blunt-end ligation into the *XbaI* site of plasmids containing PC or PCA.

METHODS. HD11/HBCI cell growth, transfection and CAT assays were performed as described²⁶. CAT activity is given as the percentage of chloramphenicol acetylated by 10 μ l of cell extract incubated for 1 h at 37 °C. Values are averages of three independent transfection experiments.

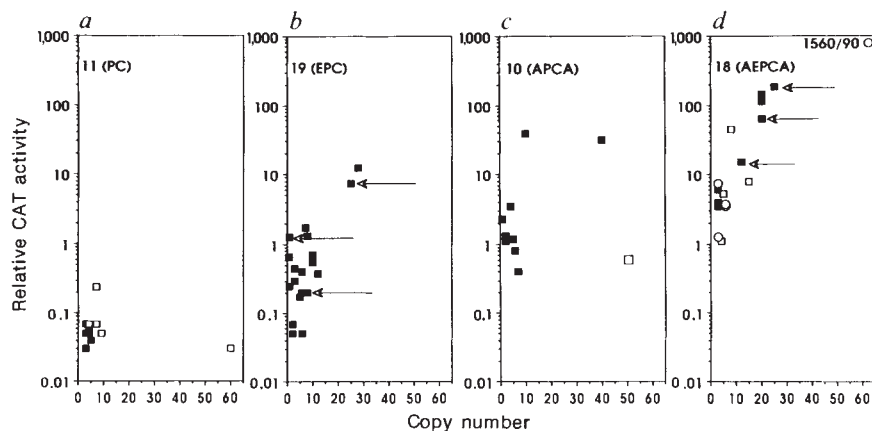
either PC or EPC minidomains confirmed the previous report of stimulation by the lysozyme-gene enhancer element²⁶. The transfection of cells with constructs containing A-elements at either or both ends of a PC element did not have a significant effect on the expression of CAT. In this arrangement, the A-elements remained neutral. When, however, cells were transfected with constructs in which the A-elements had been placed between the enhancer and the promoter, stimulation by the enhancer was inhibited. The constructs that contained an insertion, between the enhancer and the promoter, of a vector DNA fragment of roughly the same length as the A-element, demonstrated that only a small part (~40%) of the A-element inhibition

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FIG. 2 Effect of A-elements on the activity of stably integrated reporter genes. Each dot represents 1 of 58 stably transfected HD11/HBCI cell clones of two independent series of experiments (open and closed symbols). Copy number of correctly inserted DNA is plotted versus relative CAT activity. *a*, 11 clones with inserted PC-containing plasmid DNA; *b*, 19 clones of EPC-containing cells; *c*, 10 clones of APCA-containing cells; *d*, 18 clones of AEPCA-containing cells. Control experiments demonstrated that A-elements in integrated constructs containing AEPCA retained the ability to attach to the nuclear matrix or scaffold obtained from lithium 3',5'-diiodosalicylate-extracted nuclei (L. Phi-Van, unpublished results). In AEPCA-containing cells, represented by circles, the upstream A-element is in sense orientation; those cells represented by squares have the A-element in the antisense orientation. Arrows mark cell clones used for Fig. 3. The AEPCA cell clone (represented by an open circle in the right upper corner of Fig. 2*d*) has the true values of relative CAT activity 1560 and the copy number 90, as indicated.

METHODS. DNA transfection was as described²⁶. Plasmid (2 µg) containing the neomycin-resistance gene²⁹ linked to the long terminal repeat promoter of the murine-leukaemia-virus and the poly(A) signal of the thymidine kinase (*tk*) gene of the herpes simplex virus (pMPlneo), and 48 µg of the different CAT plasmid constructs were cotransfected into 2×10^6 promacrophages. 72 h later, cells were harvested, diluted (1:3) with medium containing 500 µg ml⁻¹ G418 antibiotic. After 4 weeks, G418-resistant clones were isolated. Integration of transfected DNA was analysed by Southern blotting



of 10 µg genomic DNA (*Bam*HI digestion of PC- and EPC-containing cell DNA, *Clal* digestion of APCA- and AEPCA-containing cell DNA and hybridization with probe shown in Fig. 1*a*). On average, 50% of clones showed correct integration; clones with incorrectly integrated DNA were discarded. Copy numbers of integrated DNA were determined by quantitative Southern blotting. DNA of clones with correctly integrated DNA was digested with *Eco*RI and hybridized with the probe shown in Fig. 1*a*. Signal intensities were compared with standards derived from dilution series of plasmid DNA (not shown). CAT activity was determined as described²⁶. 1 unit of relative CAT activity represents the conversion of 1% chloramphenicol by incubation with 300 µg extract protein for 1 h at 37 °C (86.6 pmol acetylated chloramphenicol per mg of protein per hour).

could be accounted for by the increased distance between the enhancer and the promoter.

To study the function of A-elements when they are stably integrated into the genome, we co-transfected selected plasmid DNA-constructs together with plasmids carrying a neomycin-resistance gene²⁹ into HD11/HBCI cells, and isolated promacrophage cell lines that were clonally derived and neomycin resistant; each of these clones represents an individual integration event. Figure 2 summarizes the result of an analysis of 58 cell lines, in which the transfected DNA-constructs were correctly integrated into the genome (Southern blots not shown). The relative CAT activity has been plotted versus the copy number of the integrated plasmid DNAs, as determined by quantitative Southern blots. The panels demonstrate the clonal distribution of genomic insertion events with PC-, EPC-, APCA- and AEPCA DNA (Fig. 2*a-d*). The comparison of CAT activities produced by the two constructs that lacked A-elements clearly demonstrates that the enhancer has an average stimulatory effect when it is in a genomic location. It compares well with the results of transient transfection assays (Fig. 1*b* and ref. 26). The CAT activities of individual enhancer-activated cell lines, however, differed considerably (Fig. 2*b*). This variability exemplifies the commonly observed copy-number independence that results from the genomic position effect.

The presence of A-elements at both ends of the inserted DNA dramatically affected the activity of the reporter gene in two ways. First, comparisons of the CAT activity of PC-containing cells with that of APCA-containing cells (Fig. 2*a, c*) and of the CAT activity of EPC-containing cells with that of AEPCA-containing cells (Fig. 2*b, d*), show that there was significant A-element-dependent stimulation of the reporter-gene activity. Second, consistently high-level CAT activity that was dependent on the copy number was obtained in AEPCA-containing cells (Fig. 2*d*), in which the inserted DNA elements resembled the arrangement of the endogenous domain of the lysozyme gene. A-elements seemed to buffer the inserted mini-domain from the influence of nearby genomic regions. Both of the A-element activities—the stimulatory activity as well as the activity that produced independence of the integration site—were found, irrespective of the orientation of the upstream A-element (Fig.

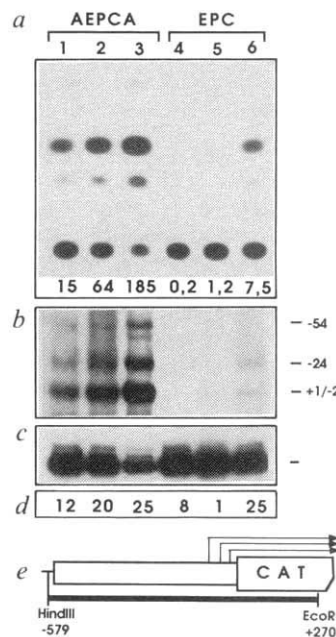


FIG. 3 A-elements stimulate transcription start at the correct promoter site. *a*, CAT assays of six AEPCA- and EPC-containing cell clones (indicated by arrows in Fig. 2). *b*, S1-nuclease mapping analysis of CAT-gene transcripts in total cellular RNA. Numbers indicate positions of start sites with respect to the main start site of the lysozyme-gene promoter^{26,30}. *c*, Signals of S1-nuclease mapping analysis of endogenous β -actin-specific transcripts. *d*, Copy number of inserted AEPCA- and EPC-containing constructs (see Fig. 2). *e*, Schematic diagram showing S1-nuclease mapping of CAT-gene transcripts. Total cellular RNA was hybridized to a 849-bp *Hind*III-*Eco*RI fragment of PC-containing plasmid (pLYSCAT2000; ref. 26) labelled at the *Eco*RI site. METHODS. CAT activity was determined as described in Fig. 2. Preparation of RNA and S1-nuclease mapping analysis were performed as described²⁶ with the following modifications: for the analysis of CAT-gene-specific transcripts, 100 µg RNA was hybridized to the DNA probe for 12 h at 43 °C; for β -actin-specific transcripts, 5 µg RNA was hybridized for 12 h at 50 °C. After hybridization the samples were digested with S1 nuclease (250 units) at 37 °C for 1 h.

2d). Figure 3 demonstrates that, also, A-elements in genomic position stimulate the start of transcription from the correct promoter site, an effect that has previously been shown for the lysozyme-gene enhancer in transiently transfected promacrophages²⁶. The signals from the quantitative S1-nuclease mapping of the reporter gene transcripts in three AEPCA-containing and three EPC-containing cell lines show that the level of transcripts parallels the activity of the reporter enzyme (Fig. 3a, b, e). The 5' ends of the reporter-gene transcripts were characteristic of the multiple messenger RNAs generated from the chicken lysozyme promoter^{26,30}.

Transcriptional stimulation of the A-elements added to the stimulatory activity of the enhancer (Fig. 2), yet A-elements and the enhancer stimulated in a functionally distinguishable manner. A-element activity can only be monitored when the element is stably integrated into the genome. In addition, the 5' A-element of the lysozyme gene does not coincide with a classical DNase-I-hypersensitive site in chromatin^{17,23}. Position independence was also found recently for inserted DNA constructs containing the human β -globin gene domain^{11,12} and was attributed to the presence of a far-upstream DNA site called the dominant control region. Here we have shown that high-level activity of the reporter gene that is independent of the position of insertion demands two types of elements—the enhancer lying 6.1 kb upstream of the lysozyme gene, and the 5' A-element of the lysozyme locus.

The two activities of the 5' A-element of the lysozyme gene in (1) sequestering functional chromatin domains and (2) increasing transcriptional efficiency, may both be functions of the structural features of the A-element that have been described previously: A-elements can attach to nuclear scaffold material and they also carry sequence motifs that are similar to the consensus sequences of DNA topoisomerase II (ref. 17). The molecular mechanism of the function of A-elements must ultimately also explain the more-than-linear (cooperative) enhancement of transcriptional activity with increasing copy number of clustered reporter genes which we observed (Fig. 2d). Whatever this mechanism, the 5' A-element of the chicken lysozyme gene may be only the first example of a new type of *cis*-acting DNA element which is very useful for increasing transcriptional activity and ensuring the correct control of randomly inserted transgenes. □

A membrane-targeting signal in the amino terminus of the neuronal protein GAP-43

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NEURONS and other cells, such as those of epithelia, accumulate particular proteins in spatially discrete domains of the plasma membrane. This enrichment is probably important for localization of function, but it is not clear how it is accomplished. One proposal for epithelial cells is that proteins contain targeting signals which guide preferential accumulation in basal or apical membranes¹. The growth-cone membrane of a neuron serves as a specialized transduction system, which helps to convert cues from its environment into regulated growth. Because it can be physically separated from the cell soma, it has been possible to show that the growth-cone membrane contains a restricted set of total cellular proteins², although, to our knowledge, no proteins are limited to that structure. One of the most prominent proteins in the growth-cone membrane is GAP-43 (refs 3–5; for reviews see refs 6 and 7). Basi *et al.* have suggested that the N-terminus of GAP-43 might be important for the binding of GAP-43 to the growth-cone membrane⁸. Skene and Virag⁹ recently found that the cysteines in the N-terminus are fatty-acylated and that this post-translational

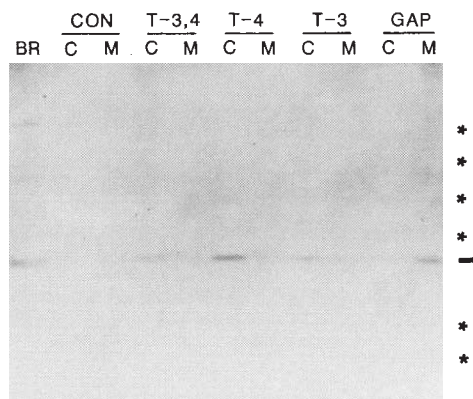


FIG. 1 GAP-43 N-terminal mutations prevent membrane association. COS cells were transfected with the expression vector pGAP (GAP) or with plasmids containing point mutations (Cys to Thr) at position 3 (T-3), position 4 (T-4) or position 3 and 4 (T-3,4). Immunoblots of membrane (M)- and cytosolic (C) fractions stained with anti-GAP antibody show that GAP-43 immunoreactivity co-migrated with purified GAP-43 protein (bar on right) and was greatly enriched in the membrane fraction. By contrast, the mutant GAP proteins were all more concentrated in the cytosol. Non-specific staining by the antibody was negligible in control fractions from CDM8-transfected COS cells (CON). A crude membrane fraction from rat brain (BR) shows immunostaining at a band corresponding to the same relative molecular mass (M_r) as the band for the GAP-transfected COS cells. The migration positions of protein standards with M_r s of 116,000 (116K), 84K, 58K, 48.5K, 36.5K and 26.6K are indicated by the asterisks.

METHODS. Point mutations in GAP-43 were generated from single-stranded DNA made from pGAP and used to alter the cysteines to threonines. The oligonucleotides GGCATGCTGACCTGTATG, ATGCTGTGCACTATGAGA, and GCAGGCATGCTGACCACTATGAGAAGAAC were used to mutate Cys 3 to Thr, Cys 4 to Thr, and Cys 3, 4 to Thr, respectively. After mutagenesis, a 200-base pair (bp) fragment containing the mutation was subcloned into a non-mutated plasmid. The sequence was confirmed after mutagenesis by dideoxy chain-termination sequencing. Other procedures are as described in Fig. 2.

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modification correlates with membrane-binding ability. We investigated the binding of GAP-43 to the growth-cone membrane by mutational analysis and by laser-scanning confocal microscopy of fusion proteins that included regions of GAP-43 and chloramphenicol acetyltransferase (CAT). We found that a short stretch of the GAP-43 N-terminus suffices to direct accumulation in growth-cone membranes, especially in the filopodia. This supports a previous proposal^{8,9} for the importance of this region of GAP-43 in determining the membrane distribution of GAP-43.

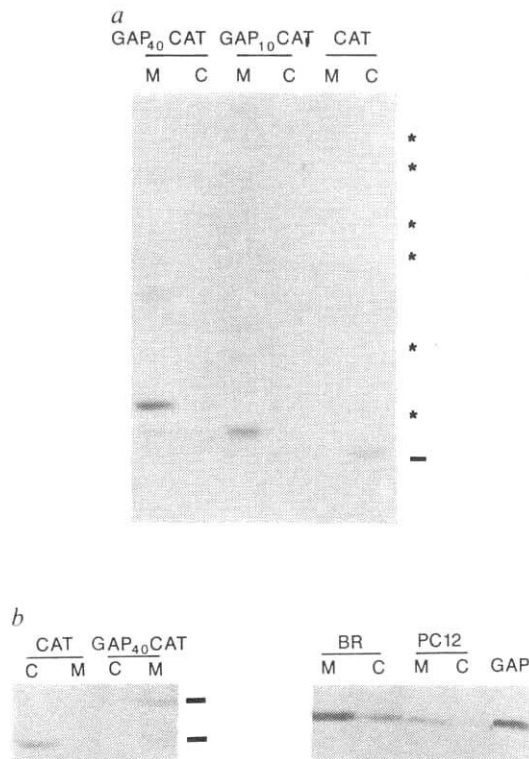
Although GAP-43 lacks the hydrophobic amino-acid sequences that are characteristic of integral membrane proteins^{9,10}, it resists dissociation by agents that usually remove peripheral membrane proteins^{9,11–14}. Hence, the mechanism of membrane binding is not clear. We have found that transfected GAP-43 binds to the membrane in COS cells and other non-neuronal cells¹⁵. The high level of GAP-43 expression in COS cells permitted preliminary screening to determine which regions were important in membrane association. Whereas membrane binding persists after deletion of a large internal segment (residues 41–189 out of 226 residues), or after deletion of the C-terminal eight amino acids and substitution of an unrelated dodecapeptide, the deletion of amino acids 2–5 markedly diminishes membrane binding (data not shown). Contained in the N-terminus are the only two cysteines in GAP-43, at positions 3 and 4, and it seemed reasonable that this unusual structure

of adjacent sulphhydryl groups might contribute to membrane binding. As shown in Fig. 1, in which cytosolic and membrane cell-fractions are compared, GAP-43 binds predominantly to the membranes, whereas replacement of Cys 3 or Cys 4, or both, by threonine, causes most of the protein to remain cytosolic. These two cysteines are palmitoylated and this correlates with membrane association in brain¹⁴. Thus, the N-terminus of GAP-43, especially its N-terminal cysteine residues, seem to be necessary for the binding of GAP-43 to membranes.

To determine whether the N-terminus is sufficient to confer on GAP-43 the ability to bind to membranes, we made constructs that encoded differing amounts of the GAP-43 N-terminus fused to a reporter peptide, and expressed them in COS and PC12 cells. We chose chloramphenicol acetyltransferase (CAT) as the reporter peptide because it is cytosolic when expressed in eukaryotic cells and is very stable. We constructed plasmids that encode fusion proteins containing either the first 10 amino acids of GAP-43, Met-Leu-Cys-Cys-Met-Arg-Arg-Thr-Lys-Gln, fused to the N-terminus of the complete CAT protein (GAP₁₀CAT), or the first 40 amino acids of GAP-43 fused to CAT (GAP₄₀CAT). As shown by immunoblotting, the CAT that was expressed in COS cells (Fig. 2a) or PC12 cells (Fig. 2b) was present only in the cytosolic fraction. By contrast, the chimaeric proteins GAP₁₀CAT and GAP₄₀CAT were membrane-associated. We were able to extract the fusion protein with detergent,

FIG. 2 Membrane association of GAP-43 and GAP-CAT fusion proteins. **a**, Chimaeric proteins with the N-terminus of GAP-43 fused to CAT associate with COS cell membranes. The constructs CAT, GAP₁₀CAT and GAP₄₀CAT were transiently expressed in COS cells. Immunoblots of membrane (M)- and cytosolic (C) fractions from each transfection were prepared using anti-CAT antibody. Note that in the CAT-transfected cells, immunoreactivity was found only in the cytosolic fraction and co-migrated with purified CAT protein (indicated by the bar). In the GAP₄₀CAT- and GAP₁₀CAT-transfected cells, nearly all of the immunoreactivity was membrane-associated and migrated more slowly than that for CAT-transfected cells, as expected for fusion proteins with an *M_r* of 4K or 10K greater than CAT. Protein standards with *M_r*s of 116K, 84K, 58K, 48.5K, 36.5K and 26.6K migrated as indicated by the asterisks. **b**, Membrane association of GAP and GAP₄₀CAT in PC12 cells. Stably transfected PC12 cells expressing CAT, GAP₄₀CAT or GAP were selected as described below. Immunoblots of membrane (M)- and cytosolic (C) fractions were stained with anti-CAT antibodies (left panel) or anti-GAP (right panel) antibodies. CAT-transfected cells (CAT) contained immunoreactivity in the cytosolic, but not in the membrane fraction, and this immunoreactive CAT co-migrated with purified CAT (lower bar to right of left panel). In contrast, GAP₄₀CAT transfected cells contained membrane-associated CAT immunoreactivity, which migrated more slowly (upper bar). Fractions from rat brain (BR) demonstrate that most, but not all, endogenous GAP-43 immunoreactivity was membrane-associated. In transfected PC12 cells over-expressing GAP-43 (PC12), nearly all of the GAP-immunoreactivity was membrane-associated and co-migrated with purified GAP-43 (GAP; indicated by far-right bar).

METHODS. In the GAP-43-expression plasmid, pGAP, the GAP-43-coding sequence replaced the stuffer region at the *Xba*I sites of the CDM8 plasmid described by Seed²⁵. The inserted GAP-43 sequence included the entire coding sequence of rat GAP-43, from the *N*allI site at the start of translation to the *Sau*3A1 site 68 bp downstream from the termination codon⁸. For the CAT expression plasmid, pCAT, the *Hind*III–*Bam*HI fragment containing the CAT-coding sequence and polyadenylation site from pSV2CAT²⁶ replaced the *Hind*III–*Bam*HI fragment of CDM8 containing the stuffer region and polyadenylation site. Plasmids pGAP₄₀CAT and pGAP₁₀CAT included sequences encoding the first 40 or 10 amino acids of GAP-43, respectively, fused in-frame with CAT in pCAT by the use of polylinkers. For transient transfection of COS cells we used DEAE-dextran and chloroquine as described²⁷. For stable transfection of PC12 cells we used a neomycin-resistance plasmid co-transfected with the plasmid of interest on a 1:10 ratio as described¹⁵. During selection of PC12 cells, 400 µg ml⁻¹ of active Geneticin (GIBCO) were used. Transient transfection of PC12 cells was by electroporation with the BioRad electroporation system using 300 V and 960 µF. After 8 h the medium was changed. Twenty-four hours after electroporation the cells were plated on poly-L-lysine-coated coverslips in the presence of 50 ng ml⁻¹ NGF and analysed 24 h later. For immunochemical assays, rabbit anti-GAP-43 antibodies were made by immunizing rabbits against four peptides including amino-acid residues 1–24, 35–53, 53–69, and 212–228 of rat GAP-43. Anti-GAP-43 antibody was affinity-purified on GAP peptide agarose. Anti-GAP antibody was bound to a resin which contained 10 mg ml⁻¹ of each peptide coupled to agarose by the cyanogen bromide method, and the antibody was eluted at pH 3.5. Rabbit anti-CAT antibodies were obtained from 5 Prime-3 Prime Inc. Secondary antibodies were obtained from Organon Teknika, Jackson Immunologicals and Vector Labs. For cell fractionation, COS or PC12 cells were scraped from 100-mm confluent Petri dishes and pelleted at 2,000g for 10 min. The pelleted cells were homogenized by Polytron in 10 mM Tris-HCl, 1 mM EDTA, pH 7.6 (300 µl per dish) and centrifuged at 250,000g for 30 min at 4 °C. The supernatant was collected as the



cytosolic fraction. The pellet was washed by homogenization and centrifugation in the same buffer, and then resuspended to the same volume as the cytosol fraction. Rat brain was obtained from 3-day-old rats and homogenized by Polytron in 10 mM Tris-HCl, 1 mM EDTA, pH 7.6 (10 ml per gram of wet-weight tissue). The cytosolic and washed-membrane fractions were prepared by centrifugation as described for the cell extracts. GAP-43 protein was purified from rat brain by a modification of the method of Andreasen *et al.*²⁸ and used as a positive control for immunostaining. The same volume of cytosolic or membrane fraction (usually 100 µl) was electrophoresed on polyacrylamide gels²⁹. Proteins were electrophoretically transferred to nitrocellulose and excess sites were blocked with 4% BSA. Membranes were then incubated for 24 h at 4 °C with 40 µg ml⁻¹ affinity-purified anti-GAP, or a 1:1,000 dilution of anti-CAT antibodies. Bound antibody was detected using anti-rabbit horseradish-peroxidase method (Vectastain) according to the manufacturer's instructions. Tetramethyl benzidine (Kiergaard and Perry, Gaithersburg, Maryland) was used as peroxidase substrate.

FIG. 3 Subcellular localization of CAT, GAP-43 and fusion proteins in transfected PC12 cells. Confocal immunofluorescence of CAT (a), GAP-43 (b), GAP₄₀CAT (c), and GAP₁₀CAT (d) in PC12 cells. Two representative fields of cells transfected with each plasmid are shown. CAT labelling is diffuse and cytosolic, whereas GAP-43 is localized to the membrane in a punctate fashion with some enrichment in the growth cones. When either the N-terminal 40 amino acids (GAP₄₀CAT) or 10 amino acids (GAP₁₀CAT) were fused to CAT, the immunofluorescent distribution resembled that for GAP-43, including enrichment in growth cones. All cells were treated with NGF for 24 h before fixation. Anti-CAT antibody was used for a, c, and d, whereas anti-GAP-43 antibody was used for b. Control PC12 cells of this variant expressed undetectable levels of GAP-43 and CAT immunoreactivity. Scale bar, 25 μ m.

METHODS. PC12 cells were transferred to poly-D-lysine-coated coverslips 24 h before immunofluorescence in the presence of 50 μ g ml⁻¹ NGF, fixed with 3.7% formaldehyde for 7 min, and made permeable with 0.1% Triton X-100 for 3 min. The samples were blocked with 4% BSA in PBS for 1 h, incubated for 1 h in primary antibody, rinsed with PBS, incubated in 0.3% H₂O₂ in PBS for 15 min (to reduce background), rinsed again and incubated for 1 h in secondary antibody. After washing with PBS several times, coverslips were rinsed with water and mounted with Gelvatol containing 0.4% n-propyl gallate to decrease bleaching. Immunofluorescence was not detectable above background when cells did not contain specific antigens or when the primary or secondary antibodies were omitted.

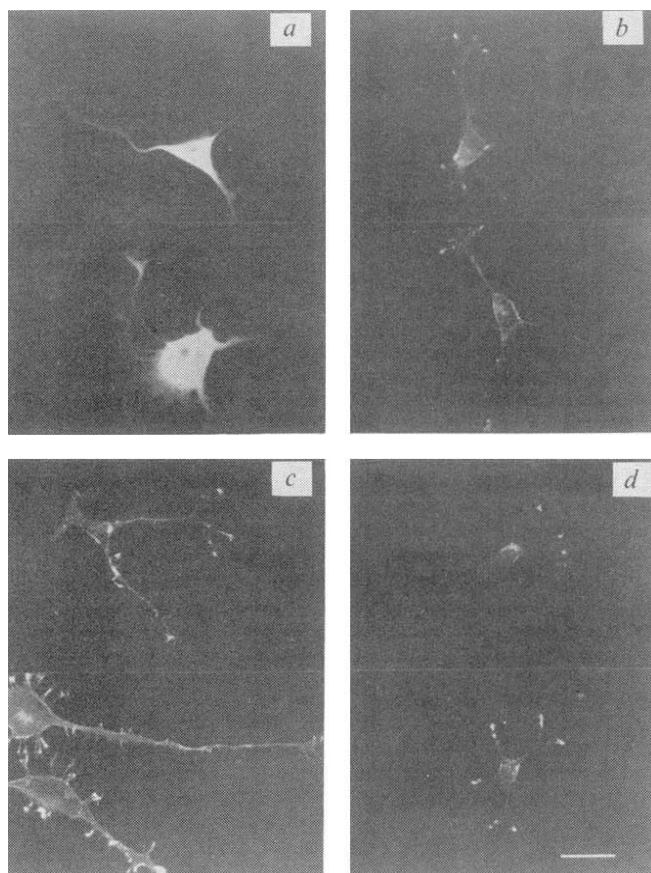
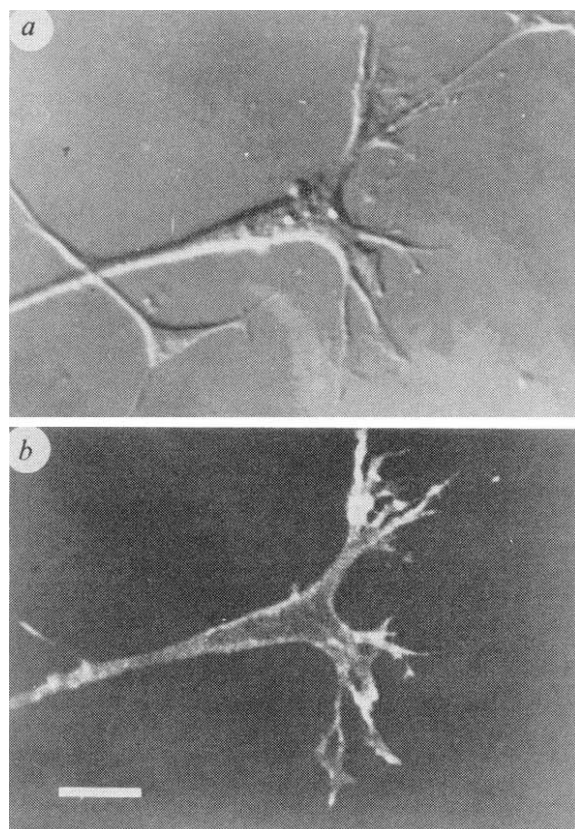


FIG. 4 Localization of GAP₄₀CAT in the growth cone of a PC12 cell. A high power comparison of PC12 cells expressing GAP₄₀CAT viewed with Nomarski optics (top) and scanning confocal immunofluorescence, labelled with anti-CAT antibodies (bottom). Cells had been treated with NGF for 7 days, one growth cone is brightly labelled, but the smaller one is not. Unequal labelling of different growth cones, even of the same cells, occurs for native GAP-43 in neurons³⁰ and did for the cells here. Comparison of the two images shows that filopodia are especially labelled. Similar results were seen for GAP₁₀CAT (data not shown). Scale bar, 5 μ m.

METHODS. For high resolution confocal microscopy, the cells were fixed with freshly made 4% paraformaldehyde and 0.5% glutaraldehyde, which was essential to preserve the fine structure of the filopodia, and then with 0.1% Triton X-100 for 3 min and 2 mg ml⁻¹ sodium borohydride in PBS for 10 min. Confocal analysis used a BioRad MRC-500 scanning confocal imaging system and a Zeiss Axioplan microscope.

but not with sodium chloride, calcium chloride or EGTA (data not shown). Thus, the nature of this membrane binding was similar to that of native GAP-43 in rat brain¹¹⁻¹⁴.

We investigated the cellular distribution of GAP-43 and the GAP-CAT chimaeric proteins in nerve growth factor (NGF)-treated transfectants of PC12 cells by confocal microscopy to determine whether the N-terminus accounts for the growth-cone enrichment of GAP-43 in neuronal cells. In this assay, CAT remained cytosolic (Fig. 3a), whereas GAP-43 was distributed in a punctate pattern with notable enrichment in many growth cones (Fig. 3b), a pattern similar to that of native GAP-43 in neurons. The N-terminus of GAP-43, which was fused to CAT,

caused the resulting fusion protein to acquire a distribution that closely resembled that of GAP-43 itself (Fig. 3c, d). Perinuclear labelling for both GAP-43 and the chimaeric protein was detected at a low level, and may have been due to localization to the Golgi, as has been observed for native GAP-43¹⁶. Glutaraldehyde fixation provided better histological preservation of the finer processes of the growth cones, and revealed that the chimaeric protein accumulated especially within filopodia (Fig. 4).

Thus, the first 10 amino acids of GAP-43 suffice to direct the cellular distribution of GAP-43. We are not aware of other proteins that have a sequence closely related to the GAP-43 N

terminus, although at least one other non-integral membrane protein that accumulates in growth-cone membranes, SCG 10 (ref. 17) has two cysteines in close proximity (at positions 22 and 24). In polarized epithelial cells, different proteins accumulate in the apical and basolateral plasma membranes^{1,18,19}, a process believed to depend on sorting signals in the protein, similar to the signals that direct traffic of membrane- and secreted proteins to their particular destinations²⁰⁻²². For epithelial cells, such signals would also recognize different regions of the plasma membrane as apical or basolateral. In neurons, the growth-cone membrane is also distinctive, although not unique, in its protein make-up. One interesting possibility is that the growth-cone membrane has binding sites that recognize and enhance the binding of the palmitoylated N-terminus of GAP-43. Although possible, it seems less likely that the palmitoylated residues interact with the lipid bilayer directly, because that would probably cause a more uniform membrane distribution for GAP-43. Along these lines, the fatty acid moiety of another acylated protein, N-myristylated VP4 of the poliovirus, has been shown by X-ray diffraction to interact with specific amino-acid residues of other viral proteins and not with the lipid bilayer^{23,24}. Because GAP-43 and GAP-CAT fusion proteins bind to the membrane of non-neuronal cells, similar or identical binding sites must be present in other cell types. However, because GAP-43 is neuron-specific, these sites would presumably be targets for different proteins in non-neuronal cells. Sorting and selective transport, however, are as likely to account for growth-cone accumulation³¹ as is the existence of specialized membrane binding-sites.

It is notable that the sorting domain of GAP-43 caused enrichment especially in many filopodia. This is the normal location of GAP-43 in these cells, as shown by electron microscopy¹⁶. Given the previous observation that transfected GAP-43 can enhance the propensity of non-neuronal cells to extend filopodia¹⁵, it will be of interest to correlate GAP-43 location with the motile activity of particular filopodia. Finally, the sequence described here may be useful in the delivery of histological markers or other agents to the growth-cone membrane. □

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Publishing and perishing?

John B. Merriman

More journals, and more expensive journals, are chasing customers with reduced spending power. Scientist themselves are in part responsible.

ONE of the great commercial mysteries of the 1980s is the production of more and more scientific journals for markets that have less and less ability to pay for them. Something is wrong. Library budgets, in Britain and the rest of the world, have not kept pace with the proliferation of new titles and price increases, and publishers seem to be running faster and faster just to keep still.

Librarians in the academic world, especially the United States, have been the most vocal in expressing their worries, but other libraries — corporate, special and so on — also have their difficulties. Subscription agents, too, are concerned, and I should first briefly explain the perspective from which I view the matter of journal publication and purchase.

Traditional links

The traditional links in the serials-information chain are author-publisher-agent-librarian-reader. The main players are the publishers and librarians, the agents being in the middle of the sandwich. Our role is to provide a full service for journal procurement, irrespective of country or language. For libraries, agents bring together all their subscriptions and offer other services (such as providing information about new journal titles, price increases and delays in publication, dealing with missing issues, and serials management systems) to help them manage their serial collections. For publishers, agents act as order consolidators. That about 80 per cent of subscriptions ordered from publishers come through agents is evidence that the role of middle-man is a valuable one. In spite of the increased numbers of titles available, however, the change of climate since the great journal bonanza of the 1960s and early 1970s has meant that subscription agents have seen their volume growth level cut as budget cancellations have taken effect.

To what extent have journal prices increased? Blackwell's Periodicals Price Index, published each year in the May issue of the *Library Association Record*, surveys 2,007 primary journals, of which 1,165 are in science, technology and medicine. In 1989 the increase over 1988 was 7.43 per cent, but this figure was influenced by beneficial exchange rates for overseas journals. The increase for British-produced journals was 10.77 per cent.

More telling is the comparison, shown in Table 1, of the average prices of the top

ten most expensive subject areas with those of five years ago. (Science and technology journals are far more costly than others; in 1989 their average price is £214, as opposed, for example, to £123 for medical journals.) In some areas, the increases are about double the increase in the Retail Price Index over the same period.

On the other side of the coin are library budgets. The University, College and Professional Publishers Council of the Publishers Association has recently produced a report on book and journal spending by British universities and polytechnic libraries in the years 1978–79 to 1986–87. In universities spending has fallen by nearly 25 per cent in real terms over that period; the loss is £17,000 per university — nearly £1 million. The polytechnics have suffered less severely, with a reduction of 9 per cent, but they have suffered nonetheless.

The main conclusions of the report are:

Expenditure on published materials, the basic resource for teaching and research, continues to decline in real terms and now, in some cases, in cash terms. The persistent and detrimental effects of funding cuts in this area of provision bring periodical cancellations and fewer book purchases and undermine the strength of research collections so vital to the support of present and future scholars.

The economic constraints on library purchasing will leave holes in the library collections that can never hope to be filled in the future. It is inevitable that in the struggle to maintain a reasonable level of book purchases, university libraries will be forced to seek more cancellations of subscriptions to add to the many thousands they have already cut since 1981–82.

Another indicator of the condition of journal publication and purchase comes from the same report. In 1987 the number of titles available rose by 12 per cent over the previous year, but the number of subscriptions for each journal fell, on average, by 10 per cent. Here is evidence

of the vicious circle whereby a new journal will probably thrive only at the expense of another. Declining circulation figures mean higher prices, and more cancellations. And so it goes on. 'Twigging', the proliferation of journals in narrower and narrower subject areas, is also involved. The overall pattern is reflected in a graph that appeared in *Learned Publishing* in January 1989 (Fig. 1 — see over).

Combined squeeze

The overall consequences of the combined squeeze from the increased number of titles, increased subscription prices and reduction in library budgets can be seen in figures from Canada — over the past ten years, Canadian librarians have cancelled 40,000 subscriptions worth some \$4.2 million. They and their counterparts throughout the world are asking whether we have reached a position in which the existence of many journals is for the benefit of contributors only, not that of readers.

The standard villains in the drama are the commercial publishers (some of them said to be more villainous than others). The accusations brought against them by the librarians are those of over-producing, over-pricing and, in some cases, of making excessive profits. Concern is also expressed at the concentration of power in just a few large companies who, it is said, hold the scientific community to ransom. But the publishers themselves cannot be the only scapegoats for a system which is characterized by the insatiable demand of the ever-growing numbers of scientists. That demand, of course, is for scientists to get their work into print, not only (no doubt) in the cause of scholarship but to produce the maximum number of papers with a view to their prospects of obtaining tenure, promotion or a grant.

In popular conception, the forces of good ranged against the commercial publishers are gathered under the banners of the university presses and learned societies. Publication of a greater proportion of journals through such bodies, the argument runs, would result in more responsible publishing and lower subscription rates.

So is the answer to the crisis to be found with the learned societies? I doubt it. Like commercial publishers, if they are to

Table 1 Average prices of journals in the top ten most expensive subject areas, 1985 and 1989

	1985	1989	Increase	
1. Biophysics, biochemistry, microbiology	£390	£552	+£162	(42%)
2. Chemistry	£379	£529	+£150	(40%)
3. Physics	£306	£450	+£144	(47%)
4. Nuclear science & technology	£329	£357	+£ 28	(9%)
5. Biology	£254	£339	+£ 85	(33%)
6. General technology	£175	£259	+£ 84	(48%)
7. Astronomy & astrophysics	£166	£216	+£ 50	(30%)
8. Polymers, paint, rubber & plastics	£137	£209	+£ 72	(53%)
9. Surgery, anatomy & physiology	£168	£208	+£ 40	(24%)
10. Electronics, electrical engineering, aeronautics	£134	£187	+£ 53	(40%)

survive they must make profit (or surplus, call it what one will) and charge for subscriptions in advance, so creating a cash reserve at the beginning of each year. As charitable institutions, in Britain they have substantial tax advantages. Moreover, many learned societies, both in Britain and the United States, still prefer to publish their journals through the commercial sector, rather than go it alone. Naïveté is unlikely to be the only reason.

At Blackwell's we have surveyed our top ten commercial publishers, and our top ten learned societies who publish independently; for the international subscription agents those two groups

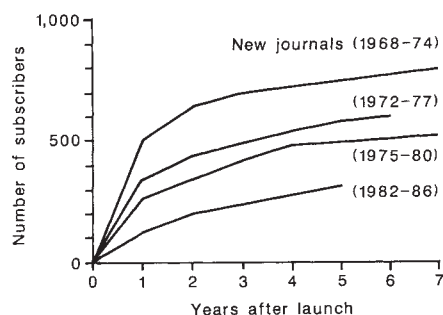


Fig. 1 Decline in circulation figures for new journals. (From A. Stevens *Learned Publishing* 2, 1-7; 1989.)

will represent a large proportion of their turnover, and their lists include a high proportion of primary journals. For 1989 over 1988 (not taking account of exchange-rate variations) the commercial publishers increased their prices by 10.57 per cent, the learned societies by 10.40 per cent. Nor are overall prices notably different — £207.78 for commercial publishers, £217.85 for learned societies.

I referred earlier to a 'crisis', which seems to me to be not over-stating the case — something, at some stage, has surely got to give in the dissemination of information through journals. Various courses of action are being advocated. More careful use of the present system by evaluating usage of individual titles against their price, replacement of expensive journals with cheaper ones, encouraging scholars not to serve on the editorial boards of journals that do not adopt realistic pricing policies and, in the longer term, the establishment of an information-clearing house to give libraries access to pricing policies, current journal rates, and proposed acquisitions and cancellation plans of other libraries. More radical would be the adoption of alternative forms of communication, in particular those employing electronics.

Meantime the demand to publish grows. No one — not least the scientific community — seems prepared to give way first. □

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New journals review 1989

THE following explanation is offered to those wishing to know the basis on which the journals mentioned in the following pages were chosen for review.

In *Nature's* previous journal supplements*, two principles determined the time criteria for a publication to be eligible for review in a particular year. First, that at least four different issues should have been published, to give reviewers a reasonable sample on which to base a judgement. Second, that most new journals start as quarterlies though they often later become more frequent.

Thus in last year's review supplement journals first appearing between June 1986 and May 1987 were considered for review. The first date was carried forward from the previous year; the second meant that four issues of a quarterly would (in principle) be available to reviewers at the time of commissioning reviews in June 1988.

Publishers and others have pointed out that journals with a schedule more frequent than quarterly could hardly count as 'new' under this system, in terms of the numbers of issues published, by the time they came to be reviewed. So for 1989 the time criteria were changed — any journal published after May 1987 and with four issues available in May 1989

was eligible for review.

Other criteria were that the journal should appear at least three times a year, and that the main language used should be English. Abstracts publications and newsletters are not reviewed. Several titles eligible for review are not covered here for one reason or another, and a list of them appears on p. 370.

The brief given to reviewers was to limit themselves to comment on the publications sent to them, and to avoid discussion of general questions of periodical publishing. Opinions expressed in the reviews are based on a sample of issues, and apply to mid-1989 at the latest. As in previous years, the preponderance of journals in the biological sciences reflects the bias of material submitted for review.

Details of editors and frequency of publication, and the subscription rates appearing at the top of each review, are given in most instances for 1990. This information is not complete in all cases, and readers interested in subscribing to a particular journal should check the rates with the publisher concerned. □

*See *Nature* 335, 459-478 (1988); 329, 357-376 (1987); 323, 359-379 (1986); 317, 293-308 (1985); 311, 309-330 (1984); 305, 477-497 (1983); 299, 491-514 (1982); and 293, 341-369 (1981).

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Planting ideas in the literature

Charlie Shaw and Phil Gates

The Plant Cell. Editor Robert B. Goldberg. *American Society of Plant Physiologists*. 12/yr. \$550 for *The Plant Cell and Plant (institutional)*; \$100 (personal).

Sexual Plant Reproduction. Managing editor H.F. Linskens. *Springer-Verlag*. 4/yr. DM 270; North America \$160.

THE recent expansion in the plant sciences, particularly in molecular biological work, has largely gone unnoticed by the front-line publications such as *Cell*, *Nature* and *Science*. The paucity of 'plant' papers in these journals has contributed directly to the failure of animal and microbial scientists to recognize the massive advances made in plant molecular biology (there are, for example, more transgenic plants than animals in existence). The void has been partly filled by the excellent *EMBO Journal*, which has always allocated a significant proportion of its space to 'plant' articles. Another, more specialized journal in this field is *Plant Molecular Biology*, but it has yet to reach real prominence.

Into the breach have stepped two new publications. *The Plant Cell* is a high-impact glossy journal, backed by the American Society of Plant Physiologists and sister to *Plant Physiology*. By the editor's own admission, *Cell* has been taken as the benchmark and the quality of reproduction, format and science are generally of a high standard. The journal was launched specifically to address the interface between molecular, cellular and developmental approaches in plants, and thus far it appears to be fulfilling this role. Most of the papers address molecular questions (the majority of them also involving some element of cell biology and development) and most concern higher plants, though the journal also aims to cover fungi and protists relevant to plant problems.

The editor is meeting his goal of rapid publication (six to eight weeks from acceptance to publication). He has also shown that he is not afraid of controversy by entering into the debate over a leading article in *Cell*, which prefaced an issue belatedly devoted to plant reviews and which was patronizing in tone and poorly researched. *The Plant Cell* has already attracted a good number of subscriptions and looks set for a healthy future, although the decline in issue size is disturbing and more colour figures and cellular/developmental papers are needed. Unless other high-profile journals increase their 'green' content, it should become the place that all plant molecular biologists look to first.

Papers on such varied topics as pollen transformation, sexual induction in *Volvox*, pollination methods for lily hybridization and a review on thermogenicity in *Arum* have appeared in the first

few issues of *Sexual Plant Reproduction*, confirming the editors' stated intention to reflect the transdisciplinary nature of research in plant reproduction. Hitherto, published work on the subject has been widely dispersed in the literature. This new journal, with an editorial board that constitutes a who's who of research workers in the field, should act as a catalyst to greater progress in our under-

Off target

Peter H. Williams

Molecular Microbiology. Editors Chris Higgins and Gary Schoolnik. *Blackwell Scientific*. 12/yr. UK £210, North America \$395, elsewhere £241 (institutional); UK £69, North America \$132, elsewhere £79.50 (personal).

THERE is something unequivocal and uncompromising, even menacing, about the title *Molecular Microbiology*. It permits no ambiguity. Not for me, it seems to say, the sweeping breadth of the *Journal of General Microbiology* or the *Journal of Bacteriology*; none of the vagueness suggested by *Infection and Immunity* or the *Journal of Infectious Diseases*. If your work is both molecular and microbiological, it says, then this is the place for you; if not, bug off.

The scope of the journal is simply and precisely what the title says it is — molecular aspects (protein structure, enzymology, immunology, gene organization and mapping, regulation, DNA topology and so on) of micro-organisms from *Anabaena* to *Yersinia* (doesn't anyone work on *Zymomonas*?), with, it has to be said, an emphasis on pathogenicity. Papers can be anything from 1,400 to 7,000 words long, although most are much less than the upper limit, short review articles ('MicroReviews'!) are invited on topics of particular current interest, and each issue bears a cover photograph (generally in colour) relevant to one of the papers it contains. Notes of up to 2,500 words are also accepted. But their format is so like that of a regular paper, and the speed of publication not significantly faster, that there seems little advantage in having a distinct category.

Acceptable manuscripts appear in print a creditable six to seven months after

standing of plant reproductive processes at every level.

New techniques in plant molecular biology have already advanced our understanding of reproductive processes, building on foundations laid by biochemists, physiologists and geneticists, and it is slightly worrying that several relevant molecular biological papers have appeared in *The Plant Cell*, rather than *Sexual Plant Reproduction*. It would be a pity if the Springer journal's potential role in integrating knowledge from widely varying experimental disciplines were to be prejudiced by molecular-orientated authors being dazzled by the high-impact format of *The Plant Cell*. □

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submission, and, unlike the journals of the American Society for Microbiology that constitute its main rivals, there are no handling charges and the first 50 reprints are free. So far, the general quality of the articles has been very high, in terms of both scientific merit and standard of writing. The quality of presentation is also excellent; the layout, with well-spaced lines and text in two columns per page, is particularly attractive and easy to read, and the cover pictures are eye-catching, if not always very original. If quality alone is the recipe for success, then this new journal is here to stay. But is it?

The problem, it seems to me, is that *Molecular Microbiology* cannot be said to fill a gap. Its scope is almost identical to that of *Microbial Pathogenicity* (another newcomer currently emphasizing molecular studies), and all the papers published so far would have found appropriate resting places in other journals of broader appeal. *Molecular Microbiology* claims on its cover to incorporate *Microbiological Sciences*, a journal that successfully disseminated the message of new technology in well-written reviews to a wide, non-specialist audience. In changing its name, this journal has also changed its nature to the extent that it no longer fulfils this function; it is now aimed unashamedly at a well-defined cross-disciplinary group which includes microbiologists who ask molecular questions and molecular biologists who happen to work with micro-organisms.

Surely, though, the real target of much of the work published here is, or should be, future practitioners of molecular methods — clinical microbiologists, for example, who may not yet be asking molecular questions, but who would be if the relevant information were intelligibly presented. I fear that *Molecular Microbiology* may have missed this target. □

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Biothis and biothat

Walter Gratzer

Chinese Journal of Biochemistry and Biophysics. Chairman of the editorial committee Lin Qi-Shui. Allerton, New York. 4/yr. North America \$265, elsewhere £280.

The FASEB Journal. Editor-in-chief William J. Whelan. Federation of American Societies for Experimental Biology. 15/yr. US \$200, elsewhere \$240 (institutional); US \$90, elsewhere \$130 (personal); US \$45, elsewhere \$85 (student).

I WAS recently told — and I do not vouch for the veracity of the tale — that an occidental author, having sent his highly rejectable manuscript to an oriental journal, received the following response: the editors, he was informed, had found his paper so overwhelmingly superior that anything their modest organ could aspire to publish thereafter would appear irredeemably unworthy. For the sake of their survival, they were therefore desolated to have to decline his offering. The point of the story is that submissions from the outside world to parochial journals are a rare enough event to throw the editors off balance, and when such papers do arrive they are unlikely to be the sort of contribution over which the international journals of the West would fight.

So the journals of the learned societies of Europe, Asia and Africa stay hidden in decent obscurity. There are exceptions (such as arguably the *Journal of Biochemistry* of Tokyo and the *Biochemical Journal*), but in general a Ruritanian biochemist with any pride or confidence in his work will want to bring it before a wider public than is reached by *Ruritanica Biochimica Acta* (especially if published in the Ruritanian language). What then, if any, is the place of national journals? Primarily, it seems to me, they are an assertion of a national scientific identity and a focus for the local research community.

The launching of the new English translation of *Chinese Journal of Biochemistry and Biophysics* (sometime *Acta Biochimica et Biophysica Sinica*) is perhaps a signal that the renaissance of biological science in China is complete — that its output can fill a respectable journal of its own. This the first four issues undoubtedly demonstrate: the coverage is representative of the biochemistry and biophysics of the 1980s and the substance is solid. It is certainly not given over, as might have been feared, to pharmacologically active products of rare oriental plants: the pages are spattered with spectra of many kinds, with restriction maps and with expression vectors. It is all respectable,

if occasionally lightweight stuff.

Everyone will wish the journal good speed. It reflects a remarkable achievement. Yet one cannot help but wonder whether what will really count for Chinese science, both inside the country and abroad, may not be the papers that the authors will want to stand beside the best in the international journals.

The *FASEB Journal* (with somewhat similar subject matter) is an altogether different proposition — its precursor, the *Federation Proceedings*, had been going for 45 years, and had, it seems, the widest circulation of any biological journal. *Fed. Proc.* published a desultory trickle of conference proceedings the year round and came to life every spring with the Federation abstracts (usually some 7,000 of them from 20,000 participants), which all biological scientists scan keenly, to see what the opposition is up to. To judge from the President's introduction in the first issue of the reconstructed model of the *FASEB Journal*, two years ago, it must suddenly have dawned on the FASEB committee that they had an existing clientele, all with

open mouths, down which could be thrust something more nutritious than conference reports and abstracts.

It was, on the face of it, a foolproof formula for success. What then have the editors made of their opportunity in the intervening years? The reviews are maintaining a decent standard with only an occasional lurch downmarket; the papers are seldom notably exciting or trendy, but those I can judge are of unexceptionable quality. But there are only five of them in each monthly issue, so the President, in seeking, as he avows, to help out *Nature* and *Science* in the struggle against the ever-advancing flood of publication, is bailing with a teaspoon.

Why only five a month? Is the editorial board rejecting hundreds in the interests of lofty standards? Is there a dearth of manuscripts? Or is it simply that they cannot afford more pages? Prospective contributors need to know. □

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Small talk

Jeffrey W. Almond

Virus Genes. Editor-in-chief Yechiel Becker. Kluwer. 4/yr. Dfl.251, United States \$126 (institutional); Dfl.150, US \$60 (individual).

IN VIRUS research during the 1980s there has been a strong emphasis on analysis of the structure and function of virus genes. This is hardly surprising, given the advances in recombinant DNA technology over this period and the realization that a detailed knowledge of virus genes is fundamental to our understanding of all aspects of virus biology. Whether this trend justifies the introduction of a new journal which aims "to serve as a platform for the publication of experimental and computer studies on genes from all virus genera and families" remains open to question. Papers dealing with virus genes already dominate most established virology journals and will continue to do so in the foreseeable future.

Virus Genes is a small journal, introduced in 1987, with most issues so far containing fewer than ten papers. The journal publishes short communications and reviews as well as full papers, and welcomes letters to the editor-in-chief. In addition, some of the issues contain editorial comment and/or a rather interesting "Viewpoint" section which deals with a contentious issue of current interest in virology, usually chosen by the editor-in-chief. Although the quality of production is acceptable, the small format means that

some figures and diagrams are cramped and difficult to read. There is also an annoying inconsistency in the layout of the references, some authors providing titles and others not.

In spite of my initial scepticism I have been mildly impressed by the quality of the papers published to date, although some of them seem to assume a wider remit than just virus genes. The prospects for success of this journal, however,



depend heavily on the quantity as well as the quality of the contributions it will attract. At the moment it is not a first-division journal because it does not contain enough material to make it a compulsory purchase for libraries. Moreover it does not have a unique position in the virology literature and is unlikely to establish one.

A glance at a random issue of one of the better virology journals revealed in excess of 40 papers, at least half of which were concerned with virus genes. Unless this new journal can attract a substantial proportion of these papers it is unlikely to establish itself in the top flight. I cannot see this happening with a quarterly publication schedule and with publication times certainly no shorter than those of its main competitors. □

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In sequence

Gunnar von Heijne

Protein Sequence & Data Analysis. Chairman of the executive editors A. Tsugita. Springer-Verlag. 6/yr. DM 610, \$353.

MOST readers of *Protein Sequence & Data Analysis* are probably not aware that the cover logo depicts the amino-terminal sequence of tetanus toxin. It is not clear to me whether some symbolic significance should be attached to this choice; but it was a rather unhappy one, in that this protein sequence is derived from cDNA data even though the journal portrays itself as the bulwark of the protein sequencers in an ocean of DNA-jocks.

The opening editorial states that the journal has three main objectives: to provide a forum for the publication of pure protein-sequence data that would not readily be accepted by other journals; to provide a medium for the advancement of theoretical sequence data analysis; and to print recent entries from the Protein Identification Resource (PIR) Protein Sequence Database as a service to scientists who do not have ready access to computerized databanks.

Judging by the number of pages devoted

to each of these objectives, the editors seem to have opted for a 1:1:1 ratio. Thus a typical issue will contain something like five full papers, one short note and 60–70 pages of PIR entries.

Overall, the scientific standard of the papers is on a par with those found in such publications as *European Journal of Biochemistry*, *Nucleic Acids Research*, *Computer Applications in the Biosciences (CABIOS)* and *Journal of Molecular Evolution*. *Protein Sequence & Data Analysis* may thus evolve to become a focal point for work dealing with the experimental and theoretical analysis of protein sequences *per se*, although papers of this kind are certainly also found in many of the main biochemical journals.

On a more critical note, I think that the large space devoted to PIR databank entries could be more profitably filled in other ways — indeed, if one can afford a subscription to *Protein Sequence & Data Analysis* one should probably first think of spending the money on a cheap PC equipped with the appropriate software and databanks. After all, it was my computer, not the journal, that identified the tetanus toxin logo for me. □

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Defence work

Fred S. Rosen

Journal of Autoimmunity. Editor J. F. Bach. Academic. 6/yr. UK £98, elsewhere \$186 (institutional); UK £49, elsewhere \$93 (personal).

Autoimmunity. Editor Terence J. Wilkin. Harwood. 4 issues/volume. \$222 (institutional); \$144 (university library); \$72 (personal).

Current Opinion in Immunology. Editors I. M. Roitt, P. Marrack and F. Alt. Current Science. 6/yr. North America \$200, elsewhere £120 (institutional); North America \$95, elsewhere £55 (personal).

Regional Immunology. Editor-in-chief J. Wayne Streilein. Wiley. 6/yr. US \$115, elsewhere \$166 (institutional); US \$80, elsewhere \$110 (personal).

DURING the past decade immunologists have made enormous progress in understanding the molecular basis of the immune response. The T-cell antigen receptors have been discovered and the genes encoding them have been mapped and sequenced. The mechanism whereby an antigen is converted into an immunogen has been elucidated. Crystallographic pictures have been obtained of class I histocompatibility molecules and they

demonstrate the crevice to which immunogenic peptides bind before interacting with cytotoxic T lymphocytes.

This has galvanized the imagination of immunologists; the hypothetical has been made real. The complex interactions of cells of the immune system are now known to involve a large array of adhesion molecules, and an unforeseen network of receptors and ligands has come to light. Amid this wealth of discovery, autoimmunity or reactivity against self remains an unsolved mystery. Many diseases such as insulin-dependent diabetes mellitus, myasthenia gravis and systemic lupus erythematosus, to name but a few, are autoimmune phenomena, and research in autoimmunity has been rapidly expanding. In consequence, we have two new specialist journals dealing with the field, one published by Academic Press, the other by Harwood.

Journal of Autoimmunity is edited by J. F. Bach of the Hôpital Necker in Paris, assisted by I. M. Roitt of London, England, C. R. Stiller of London, Canada, and N. Talal of the University of Texas. In further support is an editorial board of authoritative international figures. The journal is devoted principally to original submissions, which to date have been of a very high standard. Reports from meetings have also been included, and like the rest of the contents they are published quickly;

for example the proceedings of the First International Meeting on Immunointervention in Autoimmune Diseases appeared within six months of the meeting.

This journal appears to fill a need in an active field of research. With a modest personal subscription rate, it is good value for both researchers and clinicians. The other new entry into this arena, *Autoimmunity*, inspires no such confidence. Edited by T. J. Wilkin of Southampton General Hospital, it contains original submissions and also book reviews, invited reviews, brief meeting reports and hypotheses. But in both price and quality of the contents it does not fare well in comparison with *Journal of Autoimmunity*.

It is a daunting task to be familiar with the vast panoply of immunological literature. The publisher, Current Science, developed the innovative idea of scanning over 100 journals in immunology, publishing a list of the articles culled, and grouping them into discrete subjects such as innate immunity, immune response, immunodeficiency and so on. An authority in each field chooses several commentators who discuss the most interesting findings, and highlight and summarize the relevant articles.

Current Opinion in Immunology, the vehicle for this enterprise, succeeds only partially. There are two obvious prerequisites in such a vast undertaking. One is that the scanning of the journals must be complete and knowledgeably performed. I found several notable omissions, which led me to the conclusion that the job was not well done. The other is that the taste, authority and breadth of knowledge of the commentators have to be of the highest order. The reviews are wanting in this regard also.

Every other month two subjects are covered, such as immunity to infection and immunodeficiency or atopic allergy and autoimmunity. The format of the journal is pleasing and easy to read, and informative, original figures are reproduced. Each brief review is followed by a bibliography of relevant papers, which are graded by the reviewer as to their relative importance. After this appears a presumably complete list of the world literature on the subject discussed.

Overall supervision of the contents is not all it might be. I found three different discussions of the leukocyte adhesion deficiency in three separate issues; although this is a fascinating genetic defect, its overrepresentation is an example of imbalance in the journal's contents.

It was obviously an instance of monumental hubris to undertake this task. Its conceiver certainly ran the risk of invoking the wrath of the gods — or of those omitted in the scanning process. *Current Opinion in Immunology* is great

value for what it contains, but it would be worth twice the price if it also contained what has been omitted.

When I picked up *Regional Immunology* I had visions of reading about the niceties of immunity to kala azar in India or leishmania in Brazil. I was wrong — 'regional' here has something to do with the specialization of the immune system in different tissues such as the gut, the lung or the anterior chamber of the eye.

I was further surprised to find articles of high quality by authoritative investigators, but was hard pressed to find what was 'regional' about the contents. The journal publishes articles worthy of any good immunological journal; I suspect that the editor, J. W. Streilein of the Department of Microbiology and Immunology of the University of Miami, will find it difficult to maintain the circumscribed purpose of the journal as well as a continuous flow of high-quality original articles devoted to that purpose. □

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Neuroregulation

Jennifer Altman

Glia. Editors-in-chief Bruce R. Ransom and Helmut Kettenmann. Alan R. Liss. 6/yr. US \$180, elsewhere \$231 (institutional); US \$75, elsewhere \$105 (personal).

Journal of Chemical Neuroanatomy. Editors Harry W. M. Steinbusch and A. Claudio Cuello. Wiley. 6/yr. £160, \$290.

THE development of techniques for identifying chemical constituents of nervous tissue *in situ* has been one of the main sources of recent advance in functional studies of the nervous system. Autoradiography, immunohistochemistry and *in situ* hybridization, together with transported markers, have provided a way of relating structure to function — by revealing remarkable chemical heterogeneity in the cells of the nervous system, they have virtually added a third dimension to the study of brains.

In very different ways, these techniques are central to the two journals reviewed here. The *Journal of Chemical Neuroanatomy* has been established as a vehicle for publishing investigations using them; *Glia* is devoted to a field that owes much of its current vitality to the use of specific chemical markers for distinguishing glial cells from neurons, *in vivo* as well as *in vitro*. Both are well-produced journals, with good quality illustrations that will appeal to the dedicated followers of these subjects.

Glial cells have always been the poor relations in neuroscience. Lacking the excitement of propagated action potentials, they have often been regarded simply as stuffing between neurons. But in recent years support has come for those who have always advocated a subtle regulatory role for glial cells: they are now known to be involved in development, regeneration and perhaps maintenance of neurons; they regulate ion concentrations in the extracellular spaces of the nervous system; and they may even turn out to modulate conduction at nodes of Ranvier. They are also of clinical importance in demyelinating diseases and because of their propensity to form tumours.

These and other aspects are covered by papers in the early issues of *Glia*, which has most of the leading workers in the field on its editorial board and among its authors. Many of the contributions, which are mainly full-length research articles together with some stimulating invited reviews, are of general interest for neurobiologists. Because the workings of glial cells are clearly essential to our understanding of the nervous system, I hope the journal's title does not deter those who work on neurons from reading the work published there.

The brief of *Journal of Chemical Neuroanatomy* is wide: to publish papers giving insights into evolution, development, ageing and behaviour obtained with the large range of chemical neuroanatomy techniques now available. Despite the avowed intention of the editors to publish interdisciplinary articles that "increase the understanding of the nervous system in a comprehensive manner", the contents of the issues I have seen are limited largely to descriptive studies of localization of particular substances in a single system. Only the occasional paper meets the challenge of using techniques imaginatively in manipulative or comparative studies.

This journal thus seems likely to become a home for the vast amount of useful but rather dull descriptive work that is being produced with routine chemical neuroanatomy techniques. As long as enough people can be found to pay the rather high subscription rate, this will be a worthwhile service: because of the limited interest of each study and the need for lavish illustrations, such research is often difficult to publish elsewhere.

But to my mind, chemical neuroanatomy is one part of an integrated approach to the functional organization of the nervous system. Papers that achieve this aim should continue to be published in general neuroscience journals where they will attract far wider attention. □

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Inside trading

R.J.P. Williams

NMR in Biomedicine: An International Journal Devoted to the Development and Application of NMR Spectroscopy in Vivo. Editor-in-chief John R. Griffiths. Heyden. 6/yr. UK and Europe £155, North America \$263.

THE subject of this journal is the study of biology and its possible application to medicine (I take it that is what biomedicine is) by non-invasive NMR methods. It is not intended to cover imaging by NMR, as far as I can tell, but only the study of chemical spectra in living systems.

The technique was developed many years ago now, but naturally enough in a

NMR in BIOMEDICINE

rough and ready way. As the editor of the journal says in his introduction, "the field has undergone enormous expansion and great hopes and expectations rest on it". There is a sense of suspended judgement in this statement, which I believe is shared by many. Normally we expect an editor to state that a subject has been such an enormous success that a new outlet for papers was necessary. This journal, we are told, is required more so that "biological spectroscopists can speak to each other".

Most of us in our own corner of science also like to have a journal devoted to us — it saves us from looking around and it gives us an enhanced standing. There is a danger in this, however, and I feel that a newcomer journal would be foolishly managed if it fell into the trap. The danger is that it is irrelevant to anybody else except the specialist. *NMR in Biomedicine* needs to have some more general line. Let the main papers be as they are: about ^{13}C , ^{31}P , ^1H , ^{19}F NMR methodologies using all sorts of spectroscopic tricks. There is no harm in that. But let there be in as many issues as possible one review of what NMR used in this fashion has taught us about something (the liver, the brain, the citric acid cycle, for example) which we did not or could not know by other methods. Such reviews will reveal whether the hopes and expectations are being realized.

The editor states that NMR spectroscopy (of this kind) is at a cross-roads, and that his journal will lead it to fulfil its promise. The problem with cross-roads is that you inevitably arrive from one route but you have to know how to leave. The value of *NMR in Biomedicine* will be critically dependent on the direction taken. □

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Cell sister

Michael R. Hanley

Neuron. Editors Zach W. Hall, A. J. Hudspeth and Louis F. Reichardt. *Cell Press*, 50 Church Street, Cambridge, Massachusetts 02138. 12/yr. US \$210, Europe \$260, elsewhere \$235 (institutional); US \$94, Europe \$134, elsewhere \$114 (personal).

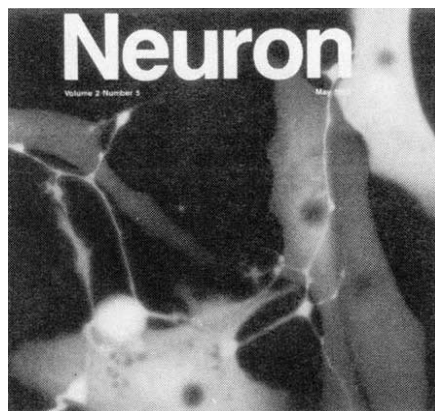
ONE of the success stories in scientific publishing has been the emergence of the biological-science journal, *Cell*. Largely, if not exclusively, the vision of one individual, Benjamin Lewin, *Cell* has become the pre-eminent forum for full-length research papers in molecular and cell biology. It is not surprising, therefore, that the publisher has set off after new challenges. The result is *Neuron*, and its aim is to be the journal in neuroscience.

Neuron publishes a combination of commissioned reviews and full-length research manuscripts. Production standards are first-rate, with high-quality reproduction of gels, photomicrographs and autoradiographs. The editorial office handles submitted manuscripts efficiently, and the turnaround time from submission to publication is often within the two-to-three months ideal achieved by *Cell* and *EMBO Journal*.

To date, the original papers have been good, but not distinguished. This may, in part, be due to the inevitable problem for specialist journals of drawing the best papers away from the broad-circulation, general-interest journals. Indeed, it remains to be seen what sort of neuroscience will be published by *Cell* in the future. For example, much of the recent research on the developmental genetics of *Drosophila* eye has widespread interest for biologists, and would perhaps be blunted in its impact if it were to appear in a parochial neuroscience setting. The generality of problems in signal transduction, cell adhesion, pattern formation, transcriptional regulation of gene expression and growth control will mean that a journal such as *Neuron* will find it progressively harder to define what belongs to its pages. But it must be said that the editors have shown a forward-looking and insightful flexibility in recognizing that points of fundamental interest to the neuroscientist may be established in some surprising experimental systems.

I am particularly impressed with the commissioned reviews which have emerged, to my mind, as the journal's clearest strength. The topics that have been covered include creation of nerve-cell lines by immortalizing gene transfer, neural-crest cell lineage, retinoids and morphogenesis, long-term potentiation, regulation of growth cones and mechan-

isms of trophic factors. These are some of the most exciting areas of neuroscience, and are subjects that profit from critical consideration of the larger biological context. In many cases, the articles break sufficient new ground to prompt the thought



that they are a new kind of primary research publication, one devoted to ideas rather than experimental results.

These successes show the influence of a tough and effective editorial board. As with *Cell*, the editors and their board are drawn from the ranks of the innovative and the progressive, and the board is not

top heavy with the identikit panel of old fogeys. This may mean that *Neuron* will be prepared to take some risks, and it will be interesting to see if its early promise of publishing provocative and stimulating papers is fulfilled.

Inevitably, *Neuron* will be judged against competitors such as *Journal of Neuroscience*, *Neuroscience* or *European Journal of Neuroscience*. Unlike the others, *Neuron* is essential for any library in the biological sciences. It is also one of the few journals for which a good case can be made for individual subscription. It is realistically (though certainly not altruistically) priced, but the main reason for recommending a personal subscription is not affordability. Rather it is that *Neuron*, like *Cell*, appears to be bound primarily by static electricity. Whoever conceived the binding of these journals has succeeded in making them disintegrate upon repeated photocopying. This trait of the *Cell Press* journals may please the publishers but annoys the scientific consumer no end. It should not be impossible to invest in a little glue. □

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Sight lines

John Cronly-Dillon

Visual Neuroscience: An International Journal for Empirical and Theoretical Research. Editor Katherine V. Fite. *Cambridge University Press*. 12/yr. UK and elsewhere £182, North America \$280 (institutional); UK and elsewhere £84, North America \$120 (personal).

NEW journals on vision appear with almost predictable regularity. They differ little from one another in style or format, which encourages the impression that their main *raison d'être* is to provide further venues for publications that help the researcher in his grant applications.

Having seen the first few issues of the *Journal of Visual Neuroscience*, I am reassured that any reservations of that sort were, in this case, ill founded. In the first issue, the editor states that "the primary goal of *Visual Neuroscience* is to bring together in one journal a broad range of excellent studies that represent the diversity and originality of contemporary research and theory dealing with the neural basis of vision". On the face of it, this seems like a *carte blanche* invitation to authors to submit a Pandora's box of contributions, that is, until one examines the list of research areas covered by the journal — comparative visual-system studies, organization and function; developmental processes and patterns, photo-

receptors and transduction; retinal anatomy, physiology, neurochemistry; subcortical visual pathways, visuomotor functions; thalamo-cortical pathways, visual cortex, perceptual mechanisms; and theoretical computational models.

This is a well-balanced selection of topics, which together probably represent the main thrust of research in visual neuroscience. As a place in which studies of them are brought together, the journal is likely to be extremely useful to active researchers who wish to keep their fingers on the pulse of a wide variety of related fields while dispensing a minimum of effort. In this respect it seems to me that the journal's appeal could be further improved by the periodic inclusion of an invited review article on a topic relating to one of the designated subject areas, and perhaps a regular editorial reflecting on notable developments in visual neuroscience.

A challenge for any new journal, even one that is well planned, is whether it is able to meet its declared intentions by attracting contributions which, in terms of quality, content and range of topics, fulfil its aspirations. Again, I was impressed on all three counts. If this standard can be maintained *Visual Neuroscience* will, I suspect, find a well-earned place on the shelf of every research worker with a serious interest in the subject. □

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From East to West

David Clark

Chemical Research in Toxicology. Editor Lawrence J. Marnett. *American Chemical Society*. 6/yr. US \$269, Europe \$280, elsewhere \$283 (institutional); US \$46, Europe \$57, elsewhere \$60 (personal).

Biomedical and Environmental Sciences. Editors Frederick Coulston and Chen Chunming. *Academic*. 4/yr. UK £77, North America \$100, elsewhere \$112, (institutional); UK £40, North America \$60 (personal).

By their very nature, toxicology and environmental science are wide-ranging subjects. They extend to all aspects of our well-being and relationship with our surroundings, and draw on virtually all scientific disciplines.

Both are fertile fields and a great attraction to publishers, who can opt for one of two strategies for their journals — the specialist or the generalist. Provided that a specialized journal finds a niche with few established rivals, it stands a better chance of being bought, read and quoted widely than a general one which has to face chillier winds of competition for quality papers. One of the journals reviewed here

is specialized, the other general (although with a unique difference).

Chemical Research in Toxicology publishes papers on modern techniques of chemical analysis applied to toxicological problems, the identification of novel toxic agents and reactive intermediates, and the metabolism of toxic agents. An invited review or perspective on some aspect of chemical toxicology appears in each issue. As would be expected of a journal of the American Chemical Society, it has been able to sustain publication of quality research papers, particularly in chemical analysis and reactive intermediates. The invited reviews and perspectives have been particularly well chosen and are of high quality.

The editors have emphasized their commitment to speed of review and publication, and so far have done very well in this respect. Only time will tell, however, if this can be maintained after the honeymoon is over. Now that *Chemical Research in Toxicology* has clearly established itself in North America, more international contributors would be welcome to ensure the journal a wider area of authority.

Biomedical and Environmental Sciences is a completely different animal and difficult to judge by normal standards. Its stated scope is very wide and embraces the biological and toxic effects of environmental pollutants on human beings and

other forms of life, with special emphasis on scientific findings in China. Indeed the editorial board state that this "is not just another journal, but a forthright decision to help overcome the Chinese Scientists' years of frustration and underdevelopment".

To this end the editors undertake to help with their contributors' English, which they have done to such good effect that the Chinese papers are indistinguishable from those written by native English speakers. The scientific quality of the articles is variable, however, ranging from standard reviews by European or American authors, such as Zbinden on animal alternatives and Munroe on risk assessment of carcinogens, to specific articles on Chinese epidemiology. Interestingly, the editors point out that because the Chinese are generally less mobile and their patterns of food consumption and pollution are

Chemical Research in Toxicology

fairly localized, there are valuable opportunities for epidemiology studies without some of the usual confounding factors.

The flow of general review articles by Westerners is likely to slow during the coming years and *Biomedical and Environmental Sciences* will then stand or fall on the quality and relevance of the Chinese contributions. One wishes a journal with the aim of opening the door to Chinese toxicology well. We must hope that recent political events in China do not close the door before the journal can make its mark. □

David Clark is the Head of Toxicology of Unilever Research, Unilever House, Blackfriars, London EC4P 4BQ, UK.

Linking up

Ian Croall

Neural Networks. Co-editors-in-chief Shun-Ichi Amari, Stephen Grossberg and Teuvo Kohonen. *Pergamon*. 6/yr. DM370.

THE arrival of this journal, described as "The Official Journal of the International Neural Network Society", accompanies the commercial emergence of neural networks as a defined topic after a long period of gestation.

The origins of the subject lie in attempts to understand the workings of the brain at all levels — biological, chemical, psychological and so on. Now there appear to be two extremes in approach. At one end there are those concerned with using the ideas inherent in the work to produce more powerful computer systems without much regard for biological fidelity. At the other we have people whose prime objective is the same as the original one, that is the construction of models which help to extend our knowledge and understanding of brain functions.

Neural Networks intends to cover the whole of this spectrum. The first two issues contain excellent introductory articles from two of the gurus of the

subject, Kohonen and Grossberg, though the connectionist point of view, reflected by Rumelhart and McClelland in their book *Parallel Distributed Processing*, is not represented. The contributions, in the first issues are of a high standard and do indeed cover the subject area well, which is most important as many traditional disciplines are involved and papers dealing with them have hitherto appeared in a wide variety of often obscure journals.

NEURAL NETWORKS

Theoretical papers come from people with a variety of backgrounds, and provide a challenge to the reader with their divergent approaches to the subject. There is rather less information on applications and comparisons with 'conventional' methods, and the emphasis is on the whole away from the biological.

Throughout, the standard of production is well up to that expected of Pergamon Press. At its present size and quality, *Neural Networks* is good value. □

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BBA 1,000

ONE of the first journals to be published by Elsevier was *Biochimica et Biophysica Acta* (BBA), which was launched in 1947. This month, the journal celebrates the appearance of its 1,000th volume.

The 1,000th volume is given over to the republication of notable papers in BBA. Accompanying them are commentaries by one or some of the original authors, setting the particular scientific finding in context and reflecting on developments since then.

Among the contributors are E. Chargaff, who writes about the paper "Studies on the Structure of Ribonucleic Acids", published in 1951; J. Brachet ("Proteines de Structure de Szent-Györgyi et Thymonucleohistone", 1947); A. Kornberg ("Enzymes Synthesis of Deoxyribonucleic Acid", 1956); S. Ochoa ("Enzymes of Fatty Acid Metabolism", 1953); C. de Duve ("Intracellular Localization of Catalase and of Some Oxidases in Rat Liver", 1960); and M. Calvin ("Intermediates in the Photosynthetic Cycle", 1956).

Over the fields

Tom Blundell

Journal of Molecular Recognition. Editor-in-chief Irwin M. Chaiken. Heyden. 6/yr. UK and Europe £149; North America \$253.

MOLECULAR recognition mediates information storage, replication and transfer in all biological systems. What then is the specific role of the *Journal of Molecular Recognition* when the subject pervades all areas of biology?

According to the information for authors, the journal is a "multidisciplinary publication devoted to research on the basic principles, characterisation and application of specific interactions in biology, biotechnology and medicine". The journal certainly meets this broad remit. It has published interesting articles by distinguished authors on immunomagnetic separation technology, the insulin receptor, cognitive features in epitopes, mimetics of β -turns, the self-assembly of neurohypophyseal hormone precursors and binding sites for bleomycin on DNA. All involve molecular interactions and recognition. But in spite of the individual excellence of the articles, the journal has yet to identify a niche in the fast-expanding fields of molecular and cell biology. The lack of a 'self-assembling and easily recognized' group of authors appears to be reflected in the very long period — June 1988 to April 1989 — between the third and fourth issues of Vol. 1.

Molecular recognition has been the topic of research in biophysical circles for many years. Fred Richards, David Phillips, Max Perutz and many others identified the importance of weak interactions involving the complementarity of surfaces, hydrogen bonds and ionic interactions in proteins two decades ago and set the scene for the theoretical analysis and quantification that has occurred since. Why then is it only in the past few years that government organizations and publishers have chanced upon the term 'molecular recognition' and exploited it with investment in new research initiatives and new journals?

The reason, in part, is the recruitment of large numbers of chemists and biologists into molecular and cell biology as a consequence of the success of recombinant DNA methodology and biophysical approaches to the three-dimensional structure of macromolecules. Much of the scientific community is new to the area even though the concepts are old. However, it is also a consequence of the emphasis on planning and themes that has recently characterized science policy even under the governments of free marketeers such as Margaret Thatcher and Ronald

Reagan. According to John Ziman, 'science in a steady state' requires more management and selection; the natural trend of science is to expand exponentially, as de Solla Price pointed out in 1963, but this is no longer possible. The new planning policies appear to have led to a proliferation of labels such as molecular recognition, biotransformations and protein engineering for activities that already have a history.

The existence of the *Journal of Molecular Recognition* thus reflects changes in the nature of scientific policy making, and also the existence of new multidisciplinary communities. The challenge for the journal is to draw out of each topic the aspects of molecular recognition which may be relevant and of interest to the wider community in this diffuse area of

science. Emphasis may be on technological advances which underpin the science, or on experimental or theoretical approaches that extend our understanding of the general principles of molecular



recognition. On the basis of the first four issues, the journal has still some way to go before it can meet these ambitious objectives that are described by the editor but not widely manifested in the material published. □

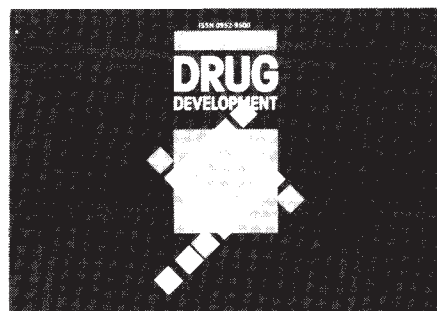
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Ill treatment

Malcolm Rowland

Advanced Drug Delivery Reviews. Editors-in-chief R. L. Juliano, G. Poste and E. Tomlinson. Elsevier. 6/yr. Dfl. 888. **Journal of Drug Development.** Editor A. D. S. Caldwell. Gardiner-Caldwell, The Old Ribbon Mill, Pitt Street, Macclesfield, Cheshire SK11 7PT, UK. 4/yr. £85, \$140.

THE dramatic discoveries arising out of cellular and molecular biology portend well for the emergence of new classes of therapeutic agents for the treatment of diseases that plague man, other animals and plants. As the editors of *Advanced Drug Delivery Reviews* correctly stress, however, these discoveries will not translate into effective treatments unless they



are matched by new strategies to achieve optimal delivery of the agents to their target sites. Innumerable promising entities have failed to reach the marketplace either, for example, because of poor absorption from sites of administration, non-specific tissue distribution with unwanted toxicity, or because of failure to penetrate the target site.

The editors of the journal have recognized that the solution to problems in drug delivery requires an interdisciplinary approach and have rightly sought reviews

that cover a wide range of areas in chemistry, biology and medicine. Emphasis has been on the newer, more advanced approaches, with a strong trend to date towards papers dealing with the placement of drug delivery in a biological context — a definite plus in my opinion.

So far all of the reviews have been of an exceptionally high technical quality, being critical, timely, thorough and well referenced. Many make absorbing reading. This is perhaps not surprising as many of the initial contributions have been written by members of a well-chosen editorial board. The technical standard is matched by a correspondingly high quality of presentation.

In addition to the established journals in the pharmaceutical sciences, several others have appeared over the past few years which would claim to cover one or more aspects of drug delivery. Among them are *Pharmaceutical Research*, *CRC Critical Reviews of Therapeutic Drug Carrier Systems* and *Cancer Drug Delivery*. Nonetheless, as long as *Advanced Drug Delivery Reviews* maintains its current quality and breadth, it has nothing to fear. It is an invaluable addition to the library of any serious investigator in drug delivery.

Drug development, from creation or discovery of an active substance, to its addition to the therapeutic armament of the prescribing physician, is a fascinatingly complex, risky but rewarding business. The high standing that society places on drug development is attested by the recent award of a Nobel prize to Sir Douglas Black for his role in the successful development of two important drugs, propranolol and cimetidine. So, when a new journal with the title *Journal of Drug Development* appears it is a matter of some interest. When, however, in its first number the editor states, albeit with commendable honesty, that he is unsure as to the scope and place of the journal,

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one can be excused for being a trifle concerned. This concern is intensified by further editorial comments and by the contents of subsequent issues.

The editor claims to offer rapid publication (which is strange for a quarterly publication) particularly of research from within the pharmaceutical industry. But there is no difficulty in publishing good research, whatever its origin, and rapidly if necessary. The contents of the journal vary from chemical analysis to (primarily) the results of clinical investigations. The articles are often short and are generally of a mediocre quality. The better ones are often reviews.

The journal has two interesting features

— each issue includes a free-ranging guest editorial, and referees are invited to append comments to the respective article. The quality of presentation is excellent and the annual subscription rates are average, but the page charges seem rather excessive given the small pages and relatively large type-size. One wonders who the target contributor can be when quotations are given for up to 50,000 offprints. Overall, there is little to commend *Journal of Drug Development* over the numerous and better journals that currently exist. □

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Flying the flag

P. F. R. Little

Genomics: International Journal of Gene Mapping and Nucleotide Sequencing Emphasizing Analyses of the Human and Other Complex Genomes. Editors-in-chief Victor A. McKusick and Frank H. Ruddle. Academic. 8/yr. UK £206, North America \$282, elsewhere \$309 (institutional); UK £78, North America \$100, elsewhere \$117 (personal).

GENE mapping was once the pursuit of a small group of enthusiasts, powered by a strong vision of what might be possible. Then recombinant-DNA technology blasted the field into a forefront of biology, the human genome initiative perhaps being its most visible feature. *Genomics* was set up in 1987 in response to this surge of activity, to provide a specialist vehicle for publication of gene mapping and nucleotide sequences.

On looking at any issue of the journal, it is immediately apparent that standards in gene mapping are very different from those in virtually all other areas of molecular genetics — not worse (or better), just different. For example, it is inconceivable to me that a report showing that a gene was *not* located on a specific chromosome or region would constitute a publishable finding in mouse or *Drosophila* research. Nevertheless such reports, as well as the normal gene/sequence localization papers, appear in *Genomics*. The reasons are not hard to appreciate — map location is definable in a positive and negative way. In consequence the journal is a very mixed read. You will frequently learn nothing from an entire issue, simply because 'your' chromosome or region or gene is not discussed.

Ultimately, a human or mouse gene map is too complex to exist on anything other than a computer database, and

much of the information in *Genomics* could be submitted directly to the database curators. Unfortunately publication in a computer database is not considered legitimate, and convention dictates that publications must follow from grant funding, or precede promotion applications. Eventually this may change and one of the main roles of *Genomics* would disappear.

Until then, the journal remains safe. It is without doubt the flagship of gene mappers. There are competitors: for example, *Nucleic Acids Research* publishes large numbers of one-page RFLP reports, but the exact usefulness of these, which often contain bald statements with no verifiable data, is rather ambiguous and the articles in *Genomics* are clearly of greater weight. Several cytogenetic and genetic journals also publish important papers. But they tend to be embedded in classical studies which, although critical to future development, are at a level less basic than gene mapping.

Probably the journal's only fault, if fault it is, is that it has not performed particularly well as a vehicle for the presentation of new technologies. Given that the whole field is technology driven, this could turn out to be a serious weakness, though recent issues show signs that the balance might be changing.

Anyone interested in gene mapping has to read *Genomics*, which is no mean achievement given that the journal was launched only two years ago. That many of its papers contain very little information, gained at the expense of much labour, is not the point; it is the construction of the whole that is important. One day *Genomics* will stop publishing mapping papers and become a collection agency for a computer database. Until that becomes an acceptable means of communication, the journal will occupy the premier publication slot in a hugely expanding area of molecular genetics. □

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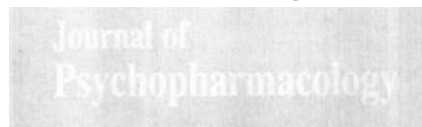
On the brain

Guy Goodwin

Journal of Psychopharmacology. Managing editor R. Maggs. Oxford University Press. 4/yr. UK £50, EEC £54, North America \$108, elsewhere £60.

Neuropsychopharmacology. Editor-in-chief J. Christian Gillin. Elsevier. 6/yr. US\$148, elsewhere \$172 (institutional); US\$96, elsewhere \$120 (personal); US\$34 elsewhere \$58 (student).

PHARMACOLOGY means far more to psychiatry than just treatment with drugs, in that pharmacological methods provide a way of analysing brain mechanisms and so of identifying abnormalities of transmitter function in psychiatric illness. Other disciplines within the neurosciences, and now also molecular genetics, are



competing to illuminate the nature of mental illness; but pharmacology, by linking therapeutics on the one hand and receptor function on the other, is still the most important of the sciences basic to psychiatry.

It is not surprising to find new journals devoted to a fusion between pharmacology, psychology and psychiatry. Furthermore, there is little doubt that there is good work to publish. The problem, as for all multi-disciplinary journals, is to maintain focus and avoid being a repository for research that is not quite good enough to be accepted by the mainstream journals in pharmacology and psychiatry. *Journal of Psychopharmacology* and *Neuropsychopharmacology* both have the advantage of originating in societies: the British Association for Psychopharmacology for the first and the American College of Neuropsychopharmacology for the second. Both are now in their second volumes; how are they doing?

Journal of Psychopharmacology aims to publish editorials, reviews, original papers, book reviews and abstracts of meetings. Its contents page does not make a clear distinction between reviews and original papers which may be because it has clearly had difficulty in attracting good-quality research reports. Indeed, the appearance of numbers 3 and 4 for 1988 in a single issue containing only two full papers with original material, looks dangerously like bankruptcy. A particular problem for the journal as a British-European publication is that two other direct competitors appeared at about the same time (*Human Psychopharmacology*,

published by Wiley, and *International Journal of Psychopharmacology*, from Clinical Neuroscience Publishers). Success may ultimately depend upon the vitality of its parent association.

Neuropsychopharmacology is a direct American equivalent to *Journal of Psychopharmacology*, not only in originating in a learned society but in having similar aims and similar composition. It has maintained a higher rate of publication of original papers, although some of these on closer inspection appear to be re-mixes of similar data published elsewhere. Nevertheless, the journal is attracting reasonable basic-science articles and good clinically orientated papers. It clearly benefits from the swing to biologically orientated research in American psychiatry. □

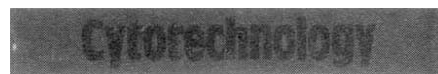
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Protein produce

W.R. Arathoon

Cytotechnology: International Journal of Cell Culture and Biotechnology. Managing editors J.B. Griffiths, D.W. Barnes and H. Murakami. Kluwer. 6/yr. Dfl.552, \$258 (institutional); Dfl.270, \$128 (individual).

ANIMAL-cell culture and related cell biology technologies are taking an increasingly high profile. There is growing demand for the complex proteins from animal cells, be they antibodies, hormones, growth factors, enzymes or vaccines, which are now often made using recombinant DNA techniques. *Cytotechnology* addresses the needs of this new field, dealing with both



International Journal of Cell Culture and Biotechnology

the pure and the applied aspects. No other journal has been directed specifically to this end.

All aspects of normal and transformed cells and hybridomas are covered. Articles range from descriptions of the use of *in situ* hybridization techniques (of cells and chromosomes) to accounts of new equipment for exploiting cells and characterizing and purifying their products. Probably not to be found in *Cytotechnology* are reports of the most recent advances in molecular cell biology, which vie for space in the fast-moving, prominent journals. Rather, here you can discover new ways of generating or activating cell lines, new growth-factor activities, more efficient culture systems and formulations for better media.

Cytotechnology contains no glossy

Special needs

C. J. Marshall

Molecular Carcinogenesis. Editors-in-chief Thomas J. Slaga and Stuart H. Yuspa. Alan R. Liss. 6/yr. US \$190, elsewhere \$241 (institutional); US \$90, elsewhere \$120 (personal).

ALTHOUGH the best papers on oncogenes and molecular aspects of carcinogenesis can still be assured of publication in the high-profile general journals, the huge increase in work in this area and the dictates of editorial fashion have created a need for specialist journals. To my knowledge, *Oncogene* and *Oncogene Research* were the first of this new generation, and these journals are now well established.

What then of the relative newcomer *Molecular Carcinogenesis*, which was started in 1988? Its editors-in-chief propose that it should be a forum for papers describing molecularly orientated research which has direct biological implications. Such an aim is highly laudable, and achieving it looks likely given the distinguished editorial board which draws in experts from many different aspects of cancer research.

On the evidence of the material that has

been published to date, the journal obviously has some overlap with *Oncogene* and *Oncogene Research*. Topics such as oncogene activation in human tumours and experimental systems, and changes in gene expression in tumours, provide common ground. There are, however, distinct differences in scope. Reflecting the background of many of the editorial board members, there is a strong representation of studies on chemical carcinogenesis. This is very appropriate, given the significance of chemical carcinogenesis in the induction of human malignancy and the importance of chemical carcinogenesis models in animals in providing reproducible systems in which oncogene activation can be studied. In contrast, there is little coverage of topics such as the biochemical functions of oncoproteins.

Molecular Carcinogenesis therefore mainly provides a perspective on how genetic changes may arise in tumour cells rather than on how those changed genes alter all behaviour. Such a perspective is clearly valuable, and the journal should provide an excellent place for the publication of work in this area. □

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advertisements — its contents are strictly down to earth. As well as research articles, it features good, short reviews, technical notes, and reviews of books, meetings and workshops. The format and layout are good. The illustrations are clear and all the print can be read without using a magnifying glass. No issues I've seen contain coloured photographs — perhaps for economy — but surprisingly this does not appear to be an obvious defect.

As might be expected of such a new, international journal, the papers published to date have been of variable quality. Most are of interest, nonetheless, and the short reviews are useful reference works. The speed of publication of papers

after receipt averages about five months, which is fairly typical for many journals of this type. There are no sections for correspondence or editorials: because the field is advancing fast, both may become desirable.

This is a practical and valuable publication for those working with animal cells in many areas. As cells are used increasingly to search for and produce useful biologically active molecules, more people will be reading *Cytotechnology*. □

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Light thickens

Bob Ford

Journal of Photochemistry and Photobiology B: Biology. Editor-in-chief G. Jori. Elsevier Sequoia. 12/yr. SwFr. 900.

THIS new journal has been created through the efforts of the European Society of Photobiology after unfruitful negotiations to share the established journal of the American Society for Photobiology (*Photochemistry and Photobiology*). The undaunted Europeans decided to join up with the old *Journal of Photochemistry*, which was renamed (apparently by committee) and split into two sections, *Chemistry (A)* and *Biology (B)*.

The scope extends to any biological phenomenon involving light. Thus in the first few issues one can find several excellent articles which illustrate the journal's wide potential. These include assessment of the use of light to treat neonatal jaundice; new data on the ultra-fast kinetics of photosynthetic energy conversion; studies on the adaptation of microorganisms to changing light regimes; predictions of the effect of the ozone hole on skin cancer; and a delightful description of the restoration of art works in the Brancacci Chapel in Florence using reflectance spectroscopy as a guide. Here is a healthy mix of articles, mostly of a high standard of presentation and of fairly wide importance.

There are, however, troubles ahead if the average handling time of papers is not reduced. Currently the delay seems to be approaching eleven to twelve months from the submission of the article to its publication. Naturally, authors who think their data is of burning importance will be tempted to go elsewhere. The problem is that the journal has been a victim of its own success — the editors have been flooded with manuscripts, leading to a long queue of accepted papers waiting for a space. The editorial board are attempt-

ing to improve matters by expanding the current volume and have negotiated a doubling of the number of issues per year to twelve.

Why should there be such a demand for an outlet in this area? One reason is that photobiology has been relatively poorly served by journals, with *Photochemistry and Photobiology* traditionally dominating the field. Also, the expanding interface between photochemistry and medicine has not, as yet, been fully explored by publishers. For instance, the development of phototherapy for the treatment of certain cancers is perhaps the most rapidly growing area of photobiology at the moment.

The new journal does cater for the more rapid dissemination of information in a News and Views section where current results can be discussed informally. This section is novel and interesting, giving the journal a refreshingly open feel in allowing the public exchange of views between contributors. In addition there are the longer invited review articles, the fully refereed research papers, an occasional book review and space for the newsletter of the society.

In general the reproduction quality is good, but the uninteresting cover design, small size and overlong name of the journal may put off the browsing reader. A further drawback is that librarians may be undecided as to its correct location. Should it go next to its sister publication in the chemistry library, or with biology and medicine? I would strongly recommend the latter — in my experience, biologists and medics rarely stray into chemistry libraries.

The journal is certainly not cheap, but with the recent agreement by the publishers to double the number of issues per year, it seems to be in good shape and looks likely to prosper. □

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Bottom lines

Peter D. Moore

Journal of Paleolimnology. Editor John P. Smol. Kluwer. 6/yr. Dfl. 476, United States \$223 (institutional); Dfl. 230, US \$108 (individual).

How can one define palaeolimnology (or paleolimnology if you come from the other side of the pond)? The subject is the study of ancient lake sediments, but there are researchers who analyse such sediments without strictly deserving the title palaeolimnologists. There are palynologists, for example, who extract fossil pollen grains preserved in the sediments in order to reconstruct vegetation history within the lake catchment and beyond. They use the fact that sediments are chronologically stratified to provide a time sequence for their work, but they are not usually specifically concerned with the history and development of the lake. The palaeolimnologist is more interested in autochthonous rather than allochthonous fossils, in the story of the lake rather than its catchment.

This new journal therefore does not deal with general environmental history. Rather it contains papers on lake levels, diatoms, chrysophytes, crustacea, caddis flies and chydorids, all of which provide information on the history of a particular lake. Some articles cover research techniques that have implications for catchment history. The inwash of clastics and

journal of paleolimnology

the accumulation of heavy metals, for example, provide a link between aquatic and terrestrial palaeoecology. Biogeographers may also need to turn to palaeolimnology to solve some of their problems, such as questions of endemism in fish (gobies from Japanese lakes find their way into the journal). Papers on techniques for the recovery of sediment cores from lakes and for the preparation of fossils from the sediments are also evidently acceptable.

Paleolimnology thus has a specialist niche to itself and has avoided an excessive intake of palaeoenvironmental papers for which there are many alternative publication media. Such specialization has advantages in terms of reduced competition from other journals, but it also has the disadvantage of a restricted market. One can only guess about the long-term success of this strategy, but I feel that the distinctive flavour of *Paleolimnology* should appeal to enough researchers to ensure its continued viability. □

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Past futures

Andrew H. Knoll

Historical Biology: An International Journal of Paleobiology. Editor E. Buffetaut. Harwood. \$186 (institutional); \$122 (university library); \$48 (personal) per volume.

PALAEONTOLOGISTS seem to enjoy two sports — predicting the imminent demise of their discipline and starting new journals. Despite numerous Cassandras, it is the second pursuit that better indicates the field's state of health. In science's modified oral tradition, palaeontologists are increasingly the keepers of systematic and morphological knowledge; the fossil record continues to provide new insights into and challenges for evolutionary biology; and as concern over global change grows, the sensitive responses of organisms to past environmental changes provide the basis for a vigorous new partnership between palaeontology and geology. To accommodate expanding research, new journals have arisen regularly over the past dozen years. The latest is *Historical Biology*.

In his prefatory remarks to the first issue, Eric Buffetaut states that *Historical Biology* will publish papers on "paleobiology, paleoecology, evolution and functional morphology, phylogeny, historical biogeography, evolutionary processes and patterns, and extinction phenomena, as well as molecular paleontology". Given that aim, one might well wonder whether the journal is to be all things to all palaeontologists (with consequently limited chances of success), or whether it will settle into a niche that differentiates it from existing outlets.

Initial results are unclear. *Historical Biology* shares several features with established English-language journals: its contents are generally if not invariably of a good standard; authors are drawn principally but not exclusively from North America and Western Europe; and its subject organisms are predominantly marine invertebrates, with a healthy admixture of vertebrate palaeontology, a few papers on skeletonized protists and plants, and virtually nothing on organic-walled microfossils. Among established journals, character displacement occurs mainly in disciplinary approach. The *Journal of Paleontology* and *Palaeontology* concentrate principally on systematics and morphology. *Paleobiology* features quantitative analyses informed by evolutionary theory. *Lethaia* also looks to biology, but in this case it is functional biology and ecology. *Palaios*, in contrast, draws its inspiration from sedimentary geology, publishing mainly on paleoecology, taphonomy and stratigraphy.

One might well argue that palaeontological studies of evolution should embrace all these methodologies, and true to its editorial claim, *Historical Biology* has taken a catholic approach. Its first issues contain such (now) standard fare as a functional analysis of pterosaur locomotion, conodont biogeography, and an essay on trilobite monophyly, as well as more unusual offerings such as a curious but interesting account of the Great Auk, an eclectic essay on plant evolution, and data on the metabolic flexibility and ecological tolerance of brachiopods. A special issue has also been devoted to mass extinctions. Although the journal as a whole includes diverse perspectives, most

individual papers still follow a single approach, much as in established journals.

It will be interesting to watch the evolution of this journal. If *Historical Biology* can encourage rigorous, multidimensional analyses of palaeontological pattern interpreted in the context of continuing changes in both the physical and biological environment, it will become a significant voice in palaeontology. As an old but newly revitalized discipline looks to the future, this, as much as anywhere, is where palaeontology's unique contributions to geology and biology will be made.

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Not so shaky

Peter D. Marshall

Earthquake Research in China. Editor-in-chief Chen Yong. Allerton, New York. 4/yr. North America \$280; elsewhere \$295. **Acta Seismologica Sinica.** Editor-in-chief Chen Yuntai. Pergamon. 4/yr. DM550.

CHINA has a remarkable record on earthquake prediction, so the appearance of two new journals consisting entirely of papers in English on the nation's earthquake phenomenology and seismological research should be of interest to seismologists throughout the world. Both *Earthquake Research in China* and *Acta Seismologica Sinica* consist of papers which have been translated by their authors from Chinese into English and made widely available by the distribution resources of Western publishing houses. From the look of the journals, it appears that they are printed and bound in China.

Earthquake Research in China is a totally new publication, the first volume of which appeared in 1987. Like *Acta Seismologica Sinica* it is run by a large editorial board, most of whom are employed by the State Seismological Bureau and are

because of the language problem. By selecting and publishing translations of papers from almost 30 Chinese-language journals, the editors hope to go at least some way towards rectifying this situation. The journal plans to take contributions on long- and short-term earthquake prediction, risk and hazard estimation and earthquake sociology (a subject which the Chinese naturally regard as very important).

Acta Seismologica Sinica is the journal of the Seismological Society of China and is in many ways similar in publishing policy to the *Bulletin of the Seismological Society of America*. It tends to contain more papers than *Earthquake Research in China* and also includes research notes. In both journals the emphasis is on seismology (though reports on other geophysical aspects of earthquake phenomenology are accepted), and both contain a mixture of theoretical and observational papers, some of which are co-authored by eminent Western seismologists. Westerners should probably not submit papers, however, because this would defeat the objectives of the publishers.

As far as I can make out it can take up to three years from acceptance for a paper to appear in *Acta Seismologica Sinica* and probably a similar time for papers in *Earthquake Research in China*, given the publishing policy. But this time lag doesn't matter too much, because the research results would probably otherwise remain unseen by non-Chinese investigators. The time taken to publish may explain why there is little evidence of use being made of the high-quality digital data from the nine seismograph stations operated as part of a vigorous co-operative programme with the US Geological Survey — it will be interesting to see how long it is before results from these stations percolate through to the journals. China operates over 400 seismograph stations and has over 15,000 technical staff employed on earthquake research, so it is not surprising that the country supports so many seismological journals.



probably responsible for peer review of the contributions. In a statement on publishing policy, Chen Yong, the editor-in-chief, says that although Chinese scientists have kept abreast of research elsewhere, the observational data and research results of Chinese scientists are rarely seen by their foreign counterparts simply

The enthusiasm of the Chinese for making results available to foreign scientists is only to be expected — after all, they have had some spectacular successes in earthquake prediction (as well as a notable failure) and have every right to be proud of their achievements. With regard to seismological data, the Chinese have followed an open policy; they report the data from their nuclear tests to the International Seismological Centre (the Soviet Union has only recently started this practice) and participate actively in the meetings of the UN Group of Scientific Experts where seismological methods of monitoring test-ban treaties are discussed.

The unique work on earthquake prediction, regional seismicity, deep seismic

sounding and crustal structure in China is worthy of wide distribution. The two journals reviewed here are of a high standard and certainly help towards filling a gap in the Earth science literature. They should be welcomed — after all seismology is a global science, with researchers world-wide depending on the free exchange of data and ideas to tackle such outstanding problems as earthquake prediction. It remains to be seen whether recent events in China perturb the previously open approach to the exchange of scientific information; it would be most unfortunate if they did. □

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Sixties style

John B. Thornes

Geomorphology. Editor-in-chief M. Morisawa. Elsevier. 8/yr. Dfl. 2928.

IN ITS early days, at the latter part of the last century, geomorphology was rooted in both geology (in North America) and geography (in Europe). The geologists were mainly interested in what the subject could contribute to Earth's history at a time when they were less preoccupied with geochemistry and geophysics. The geographers saw it as the key element in understanding the contemporary landscape, and sought to find out how processes such as weathering, erosion and tectonic activity interacted with rocks to produce the landforms we now see.

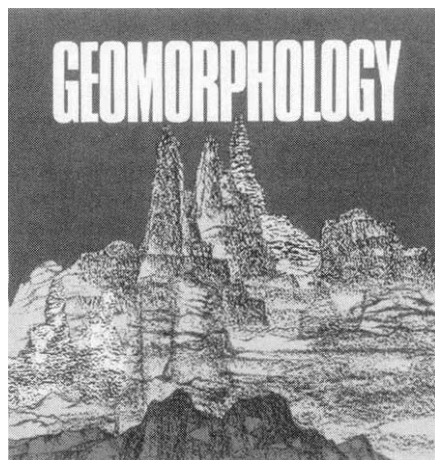
Since the late 1950s the historical and mainly descriptive element and the process element have gradually moved further and further apart, each spawning its own specialist journals for historical (mainly Quaternary) studies and for investigations of Earth surface processes and landforms. The former has allied with sedimentary geochemistry and palaeobotany, the latter with hydrology, soil physics and chemistry, and hydraulics.

Geomorphologists therefore have a large number of publication outlets; not only are there the three main journals covering the field, but plenty of others dealing with geology, hydrology, geography, soil science and (more recently) environmental issues. So this newcomer has to find a place within a highly competitive environment.

The route the editor has taken is to re-occupy the ground lost in the 1960s by concentrating on the application of contemporary processes to solving problems with a historical dimension. There is also a reassertion of the importance of geology as an underpinning of most if not all

geomorphological problems. The methodology has so far emphasized the empirical and descriptive rather than the analytical, and the bridge with Quaternary research has been quickly and effectively re-established. The journal has a North American provenance and market at a time when the main research and publication thrust had shifted to Europe and European journals.

For all these reasons *Geomorphology* has a good chance of success. Too much of intellectual value has been discarded in the past two decades, leaving a disaffected group without a focus of publication. The other main journals, *Catena*, *Earth*



Surface Processes and Landforms and *Zeitschrift für Geomorphologie*, each excellent in its own area, failed to attract this wider spectrum of interest.

The main danger, already evident in some of the papers, is that in addition to picking up the abandoned intellectual issues of the 1960s, *Geomorphology* may pick up the abandoned methodologies of the same period. If Morisawa can avoid this pitfall, the journal seems likely to attract a wide readership. □

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Entomological action

John Brady

Journal of Insect Behavior. Co-editors William J. Bell and Thomas L. Payne. Plenum. 6/yr. United States \$135, elsewhere \$160 (institutional); US \$42.50, elsewhere \$50 (personal).

HITHERTO there has not been a journal devoted to insect behaviour. On the other hand, there have been others that for years have contained the bulk of the literature on the subject: notably the *Journal of Insect Physiology*, *Physiological Entomology* and *Comparative Physiology*. These are all of European provenance, however, and are concerned more with the mechanisms of behaviour — what the Germans significantly call *Verhaltensphysiologie* — than with its description.

The *Journal of Insect Behavior*, by contrast, is intended to cover almost everything behavioural that can be examined in terrestrial arthropods, including descriptive studies, social behaviour, orientation, learning, behavioural modelling and all that which is nowadays called 'behavioural ecology'. It is also an American publication, with half of its editorial board from US institutes and most of its papers from there, too. So, if its expansion from four to six issues a year from its second volume is any indicator, it is evidently fulfilling a need.

Thus far, the quality of its contents have been generally good, although the occasional "Short Communication" (always a doubtful scientific bet) has been distinctly weak. Happily, the bulk of its coverage has not yet seriously duplicated its older rivals' interests in behavioural physiology. For those mainly concerned with that area, it is to be hoped that this lack of overlap will continue.

At the end of the day, however, it is an editor's job to do more than just publish good science: he also has a duty to maintain the communication of science at a reasonable level of literacy. Without this check and control, scientific communication can degrade into the unreadable, telegraphic and ambiguous. Sadly, this task seems to be taking an increasingly secondary role in scientific editing. Perhaps that is because of the pressure to get out more and more papers faster and faster; or is it just that no one cares any more? In this respect, the literacy of the editors' introductory statement to Vol. 1 of the *Journal of Insect Behavior* is not particularly encouraging. □

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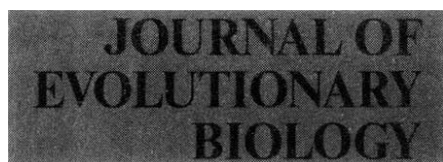
The European identity

Jerry A. Coyne

Journal of Evolutionary Biology. Managing editor S. C. Stearns. Birkhäuser. 6/yr. Swiss franc 288, elsewhere \$192; Society members SwFr. 70.

ONE of the striking aspects of evolutionary biology is its dominance by anglophones. Although the Society for the Study of Evolution is supposed to be an international organization, 92 per cent of its members are from English-speaking countries and its annual meetings are always held in North America. This has led some evolutionists, particularly in continental Europe, to feel that their contributions are discounted for linguistic reasons.

To provide a more international forum, the European Society of Evolutionary Biology and its official organ, *Journal of Evolutionary Biology*, were created in 1987. According to Stephen Stearns, its



energetic editor, the journal also seeks to fill a void by "bringing together a set of specialties that have not previously shared a common arena for discussion" (Vol. 1, page 2), including systematics, molecular evolution, development, palaeontology, genetics and ecology. Each issue contains about five papers or notes, a few short book reviews and an occasional transcript of a talk from the annual meeting.

After two years, the journal has reached its first goal, but not its second. Thanks to a board of young editors from 13 countries, the quality of the papers is high, nearly equal to that of *Evolution*, by far the best journal in the field. There is also a good geographical mix of authors, more than 40 per cent of them from non-English-speaking countries (*Evolution* has only a third as many). Although rapid publication is not terribly important in this field, the journal is faster than most, with nine to twelve months from submission to print. The illustrations are excellent, and the format (except for the garish red-and-green cover) attractive.

But the gap that *Journal of Evolutionary Biology* seeks to occupy does not exist. For many years it has been comfortably filled — indeed, choked — by journals such as *Evolution*, *The American Naturalist* and *The Biological Journal of the Linnean Society*, all of which cover a

variety of evolutionary topics. In addition, the excellent *Trends in Ecology and Evolution* reviews developments in every area of population biology. Except for its high proportion of European authors, *Journal of Evolutionary Biology* is indistinguishable in content from *Evolution*, both of them having many papers on evolutionary quantitative genetics, sex and mating systems, the evolution of development, and life-history optimization. It is hard to see how, short of commissioning interdisciplinary review papers, the editors could create a new and broader perspective.

Unlike species, journals inhabiting the same niche may coexist indefinitely. It is possible that the increasing number of papers in the field or the more European character of the journal will keep it alive. It is also possible that the encouragement of contributors from more countries will elicit novel points of view (this has not happened yet). The editors should, however, fatten up the journal if it can be done without sacrificing quality. Members now pay the equivalent of \$2.25 per paper (\$4.75 for libraries), nearly seven times the cost of each paper in *Evolution*. This disparity may decrease when more evolutionists realize that *Journal of Evolutionary Biology* is an attractive alternative to older publications. □

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Catastrophism

John Whittow

Natural Hazards: An International Journal of Hazards Research and Prevention. Editors M. I. El-Sabh, G. Schneider and Y. Tsuchiya. Kluwer. 4/yr. Dfl. 272, North America \$127.50 (institutional); Dfl. 125, North America \$59 (personal).

It is hardly surprising that the beginning of the International Decade for Natural Disaster Reduction should be accompanied by the appearance of new journals. Although the topic has been extensively covered by books, both popular and academic, it has hitherto been poorly served by faster-moving specialist media.

The exception has been *Disasters: The Journal of Disaster Studies and Management*, which has run for more than a decade. As its name suggests, however, this publication is geared primarily to disaster relief and management research rather than to analysis of the physical processes involved (though such studies are by no means excluded). So one has been forced to search through a huge variety of engineering, atmospheric science, Earth science and occasionally social science journals to discover detailed information

on natural hazards and their effects.

One should welcome, therefore, the appearance of two new journals in 1988 — *Disaster Management: An International Journal of Contingency Planning for Large Scale Emergencies*, which is published by Argus Press, Redhill, UK, and *Natural Hazards*, the subject of this review. The two differ somewhat, in that the first deals largely with human-induced incidents, while the second is mainly (but not exclusively) concerned with natural events.

Natural Hazards is the journal of the International Society for the Prevention and Mitigation of Hazards, a society spawned from the Rimouski International Symposium on Natural and Man-made Hazards, held in August 1986. The impressive list of 29 members on its editorial board is drawn from a variety of disciplines in no less than 17 countries. The journal has already been recognized as a forum for discussion and as having fostered a beneficial exchange of information between Earth scientists, engineers, planners and other policy makers. The editorial policy is to carry research articles on the physical processes, on the nature of their impact and on how such impacts may be ameliorated both by structural and non-structural means. Moreover, contributions on man-made and technological hazards are invited; it will be interesting to see the results, for there has been a noticeable increase in industrial hazard research during the past decade.

If the four issues of Vol. 1 are anything to go by, the response to the catholicity of the editorial approach has been reasonably successful. A wide selection of articles has been published, ranging in subject matter from earthquakes, volcanoes and debris flows to tsunamis, storm surges and coastal erosion. To illustrate the journal's even wider brief, there has also been a paper on simulation of oil-slick movements.

The 400 pages of the first volume include 24 articles varying between eight and 22 pages in length. The remainder of the volume is taken up by a valuable "Chronicle" that previews forthcoming conferences, book reviews, a list of recent publications and a correspondence section. The quality of the contributions, though uneven, is acceptable and in general the journal design and production create a favourable impression.

The same cannot be said, however, for the quality of the cartographic material. There is a woeful inconsistency of style and worrying variation in the legibility of the diagrams; there is no place for free-hand lettering and graphics in an international journal launched at the end of the 1980s. □

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All about a continent

Stephen Moorbath

Journal of South American Earth Sciences. Executive editors Carlos E. Macellari and Norman J. Snelling. Pergamon. 4/yr. DM190.

ON principle, I have been sceptical about the recent proliferation of regional Earth-sciences journals. It seemed rather old-fashioned to break the Earth sciences up into separate regions rather than separate disciplines, as most conventional journals do. Yet, faced with *Journal of South American Earth Sciences*, I have had second thoughts. Why should one not apply the full panoply of modern Earth-sciences techniques and disciplines to a major geographical sector of the Earth's crust in which most known geological environments are so splendidly represented? It might provide an orderly,



manageable approach, both from scientific and editorial viewpoints.

Inspection of the four issues of Vol. 1 (1988) of this well-produced large-format journal dispelled the notion that it was simply a decent burial ground for descriptive papers on regional geology in the old style. It genuinely deals with all branches of modern Earth sciences as applied to South America (as well as Central America, the Caribbean and the Antarctic Peninsula). It covers the entire range of igneous, metamorphic and sedimentary environments from Archaean to the present, as exemplified by Precambrian cratons, mobile belts, sedimentary basins, young arcs and Quaternary rocks, all of which abound in South America. In addition, economic geology is represented by papers on ores and hydrocarbons — there have not been many such contributions in Vol. 1, but no doubt there will be more in the future.

The authorship of the 32 papers in

the first volume is truly international, including well-known researchers from economically developed countries working in close collaboration with members of some of the principal university departments and geological survey organizations of South America, where the Earth sciences are taken very seriously indeed.

As well as full-scale scientific papers, the journal publishes short notes, discussions and book reviews, as well as relevant conference and workshop reports. Summaries of new developments in already established regional projects, as well as new initiatives dealing with the geology of the continent, will be summarized and presented on a regular basis. The main language used is English; papers in Spanish are acceptable, although there

have been none in the first four issues. Abstracts appear in English and Spanish.

Altogether, then, the overall quality is high and the journal will be an important reference for all workers interested and engaged in, or embarking upon, geological research of any kind in South America and surrounding regions. Shrinking library resources may threaten its existence, which will also depend on maintaining and improving the already promising quality and range shown by papers in the first volume. But it deserves to succeed, and I hope that it and other regional Earth-sciences journals can establish a viable foothold. □

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Them and us

Juliet Clutton-Brock

Anthrozoös: A Multidisciplinary Journal on the Interactions of People, Animals, and Environment. Editor Andrew N. Rowan. University Press of New England. 4/yr. US\$50, elsewhere \$60 (institutional); US \$40, elsewhere \$50 (personal).

THE aims of *Anthrozoös*, which was launched in 1987 by the Delta Society, are to promote multidisciplinary studies on the interactions of people, animals and the environment. Articles in the journal cover subjects as diverse as ethology, psychology, veterinary medicine, archaeozoology, anthropology, and linguistics.

Although one of the most anciently observed characteristics of human beings is their ability to interact with other species, the study of these affiliations is still generally perceived as a peripheral subject for scientific research. The need for people to have animals as companions, particularly in large cities, may be passively accepted by those in authority but there is little realization of the integral role played by pets in society. For example, it is still considered 'neurotic' for a lonely person to grieve over the loss or death of an animal. Similarly, the health benefits that accompany the keeping of pets are largely ignored by the medical profession and those who look after the elderly and disabled.

The intention of *Anthrozoös* is to draw together the scattered publications in this field and to provide scholarly and informed articles on all aspects of animals and human society. The first paper, by R. Lockwood and K. Rindy, in Vol. 1 No. 1, was an analysis of the controversy on the breeding of pit bull terriers, the aggressive natures of which are becoming an increasing danger. Many other socially important articles have been published in the

succeeding issues, dealing with topics as varied as equine behaviour problems (K. A. Houpt), zoos today and tomorrow (M. H. Robinson), and an evaluation of a pet programme in prison (A. Katcher, A. M. Beck and D. Levine).

It perhaps requires less intellectual courage to launch a journal such as this in the United States than it would in many other countries where there is still a certain inhibition about studies that can be labelled 'sentimental' or 'anthropomorphic'. But the time is right for a new, scientifically rigorous approach to understanding the many facets of human associations with animals and this new journal provides a professional focus for publication of the results. Such a wide-ranging journal would be improved, however, by a greater international input, and the European editor and members of the editorial board from countries outside the United States should work to achieve this.

Anthrozoös contains a balanced combination of refereed papers, comments, reviews, letters and reports of meetings. The journal is expertly produced, and the contributions have evidently been carefully edited to be readable and free of jargon. If a wide readership can be established, the journal should lead to a greater understanding of the interactive behaviour of humans and animals and should become a powerful influence in the cause for animal welfare. □

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Autumn books

The next review supplement to be published in *Nature* will be Autumn books (16 November).

Among the books to be reviewed are *Dangerous Diagnostics: The Social Power of Biological Information* (by Dorothy Nelkin and Laurence Tancredi), *Wonderful Life: The Burgess Shale and the Nature of History* (Stephen Jay Gould), *The Making of Andrei Sakharov* (George Bailey) and *The Mind Has No Sex?* (Londa Schiebinger).

Geophysicists wanted

Andrew S. Mackenzie

Journal of Petroleum Science & Engineering. Editors G.V. Chilingarian, E.C. Donaldson and K.J. Weber. *Elsevier*. 8/yr. Dfl.526.

THE aim of this new journal is "to bridge the gap between petroleum engineering and petroleum geology". The editors have attracted excellent contributions from the different corners of petroleum science, most of which can be understood by laymen with a general knowledge of the oil industry. The articles are published within a year of submission, and Elsevier offer a printing format and standard which is similar to their other Earth-science journals.

In the first issue we learn that the most economic solution for the Prudhoe Bay gas cap is to turn it into alcohol. The previous paper told us that unless the

of oil and gas arise because although the engineers' techniques are capable of handling complex descriptions of the rocks between wells, the geologist can deliver only imprecise descriptions. Resolution of this problem is more likely to come from improving the seismic image than from further application of conceptually based geological techniques.

All budding petroleum polymaths should look at this journal. I hope that it will stimulate constructive collaboration (and that the editors will include some geophysicists). □

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Out of the toy box

Mike Sharples

International Journal of Expert Systems: Research and Applications. Editor-in-chief Mehdi T. Harandi. *JAI, Greenwich, Connecticut, and London*. 4/yr. US\$135, elsewhere \$155 (institutional); US\$75, elsewhere \$95 (personal).

AI & Society: The Journal of Human and Machine Intelligence. Editor Karamjit S. Gill. *Springer-Verlag*. 4/yr. DM224, \$135.

FIFTEEN years ago Edinburgh University had a robot named Freddy, which consisted of a rotatable arm that could grasp objects placed on a motor-controlled table below it. Cameras gave two views of the table to enable objects to be positioned below the arm. A number of programs were written to demonstrate Freddy's abilities. In one of these demonstrations, toy blocks were scattered on the table and Freddy would locate each block in turn and add it to a stack in the centre of the table. Lighting had to be carefully set to avoid spurious reflection, imprecisions in the vision-arm coordination meant that the blocks formed a somewhat disorderly stack, and once it grew to about six blocks high the movement of the table caused the whole stack to tumble over.

Freddy's performance epitomized artificial intelligence (AI) in the early 1970s: a set of rather precarious tricks with toy objects. During that period work was also underway to provide the theoretical foundations to AI, by means of extended logics, simulations of human reasoning and powerful programming tools, but the dream of a 'general problem solver' had been ousted by the hard slog of crafting representations of knowledge to suit each new domain.

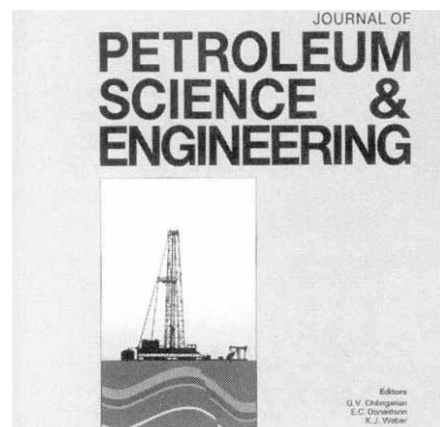
Fifteen years on there is still no general problem solver, and AI still has no sound

theoretical base, no methods of predicting the behaviour and guaranteeing the reliability of large systems. What occurred in the meantime was the discovery that the same techniques that were used to develop the 'toy' programs of the 1970s could be used to build commercially successful systems for the 1980s. Some tasks, such as the analysis of production problems in fibreglass manufacture, and the medical diagnosis of strokes, can be regarded as bounded problems for which rules can be specified to produce plausible inferences from available data. Such 'expert systems' can provide valuable assistance to human decision making, and the number of commercially deployed expert systems has risen from 50 in 1987 to 1,400 in 1988.

The *International Journal of Expert Systems* serves the growing community of expert-system designers and users. Its editorial board, although drawn mainly from academic institutions, leans markedly towards North America, where most of the commercial applications are to be found. The journal offers rapid publication and consists almost entirely of long papers of good quality and presentation. Without straying too far from the central theme, the first five issues contain articles on natural-language processing, logic programming, inexact reasoning, machine learning and cognitive modelling.

The journal's main limitations are those of expert-systems research in general: there is no guarantee that techniques devised to solve one problem will be of use in another. Tasks that are superficially similar may require very different methods of reasoning; it is unlikely that the belief mechanism for the expert system to diagnose faults in fibreglass production will be much use to the developers of the stroke consultant.

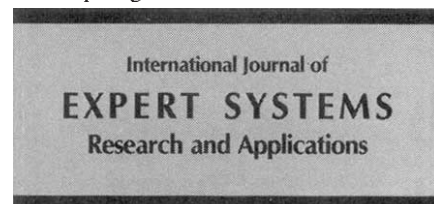
Now that AI has become commercially lucrative, and expert systems are appearing in banks, boardrooms and battlefields, the field needs to be subjected to reasoned appraisal by sociologists, economists and anthropologists. That is the aim of *AI &*



microbes in waters injected into reservoirs are dissolved with bleach they block the pores of the rock. Other issues juxtapose a paper on clay minerals with a theoretical treatment of non-newtonian flow in porous media, and one on limestone porosity with an account of the problems of injecting fluids through highly deviated wells. The most recent of the issues available for review concentrates on enhanced oil recovery.

Although I found the diversity of contributions to be stimulating, I felt that any of the papers could have appeared in more specialist journals: there is little evidence of cross-fertilization as yet. Reading the journal is like eavesdropping on the early stages of one of those parties where the host invites his friends from work and the hostess her friends from work. Everyone is introduced at the beginning, then they re-congregate in their original cabals.

The editors have left a crucial set of people off their guest list — the geophysicists. Some difficulties in the exploitation



Society: "to provide a forum for exploring the likely effects of these new technologies, and for debating policy and management issues". The journal is philosophical in approach and European in outlook, with half the advisory board from Britain. Several themes have emerged from the first two volumes: the weakness of cognitivism as a foundation for artificial intelligence; the location of computing practice within a culture; the apposition

PROTEIN-NUCLEIC ACID INTERACTION

Volume 10 Topics in Molecular and Structural Biology

Edited by W. Saenger and
U. Heinemann.

The book contains articles highlighting the rapidly evolving field of protein-nucleic acid interactions. Individual chapters demonstrate the diversity of biological systems to which protein-nucleic recognition is of central importance.

There are chapters on the interaction of small proteins with DNA and RNA as well as on chromatin organisation. Care has been taken to include accounts of many different experimental approaches to studying the subject. All articles are written by scientists actively involved in the research they describe and considered experts in their respective research areas. The book is well-illustrated and contains extensive bibliographic information to provide easy access to the original literature.

Contents DNA-Protein Interactions in the Regulation of Gene Expression - P.H. von Hippel (University of Oregon, Eugene, USA) and O.G. Berg (Uppsala University, Sweden); / Structures of Protein-Nucleic Acid Complexes in Solution by Electro-optical Analysis - D. Porschke and J. Antosiewicz (Max-Planck Inst., Göttingen, FRG); / NMR Studies of Protein-DNA Recognition. The Interaction of LAC Repressor Headpiece with Repressor DNA - R. Kaptein, R. Boelens and R.M.J.N. Lamerich (University of Utrecht, Holland); / The Single-stranded DNA Binding Protein of *Escherichia coli* - J. Greipel, C. Urbanke and G. Maass (Medizinische Hochschule, Hannover, FRG); / Protein-Nucleic Acid Interaction in Tobacco Mosaic Virus - G. Stubbs (Vanderbilt University, Nashville, USA); / Structural and Functional Studies of Ribonuclease T1 - U. Heinemann and U. Hahn (Freie Universität, Berlin, FRG); / Tet Repressor-Tet Operator Interaction - W. Hillen and A. Wissmann (Inst., Mikrobiol. and Biochem., Erlangen, FRG); / Structure and Condensation of Chromatin - M.H.J. Koch (DESY, Hamburg, FRG); / Conclusion / Index

July 1989 £40.00 214pp 234 x 156mm
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AI & SOCIETY

The Journal of Human and Machine Intelligence

of machine-centred and human-centred technology. Book reviews, news of conferences and recent projects, and an "Open Forum" containing short contributions, provide a variety of form.

Contributors from such diverse backgrounds as anthropology, sociology and theatre ought to make for an interesting and stimulating interchange of ideas, but they lack a common language. The authors cannot use the language of their own discipline, because the readership of

AI & Society is broad, while the language of AI is too restrictive to embrace its own criticism. The most successful articles are those that offer a focused critique of AI or cognitivism, such as Dreyfus's dismissal of the Socratic assumption that intelligence consists of solving problems by following rules and Pullum's scathing attack on the US Department of Defense's Strategic Computing Initiative (SCI). A major aim of the SCI is to develop autonomous military robots and weapons-control systems. The early prototypes have displayed all the limitations of Freddy the robot; but these AI systems of the 1980s no longer inhabit a toy world. □

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Harmony and discord

Robert Temple

International Journal on the Unity of the Sciences: Interdisciplinary Studies of Knowledge and Values. Editor Marcelo Alonso. *International Conference on the Unity of the Sciences*, GPO Box 1311, New York, New York 10117. 4/yr. North America \$25, elsewhere \$39.

Science as Culture. Editor Les Levidow. *Free Association Books*, 26 Freegrove Road, London N7 9RQ. 4/yr. £35 (institutional); £20 (personal).

To scientists the sciences are often known for their *disunity*, so a new journal dealing with interdisciplinary studies is welcome. Despite its clumsy title, *International Journal on the Unity of the Sciences* turns out to be a superior forum for the discussion of questions affecting not only the sciences but society as a whole.

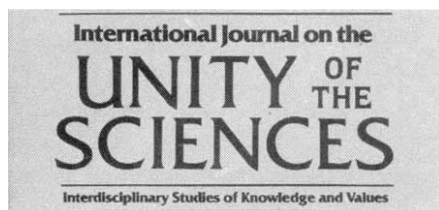
The editors have set themselves a bold agenda and adhere to it with admirable determination. The discourse is at a high level. Figures such as Eugene Wigner, Max Jammer and F.A. von Hayek have contributed articles, which, in general, are intended to combine authoritativeness with accessibility to those outside the particular field under consideration. The journal is proving very successful in meeting this aim. After only a year of publication, it has to my mind become one of the most stimulating of periodicals dealing with science in a wider context.

Here are to be found thought-provoking contributions on issues ranging from *in vitro* fertilization to chaos theory as applied to economics, taking in the world food problem and all manner of ethical and philosophical questions along the way. Many of the ideas and insights are unusual, and the arguments convincing.

On the other hand, one must mention the undue number of typographical errors which appear.

Science as Culture is a different matter entirely. Although the proof-reading can't be faulted, the contents can.

The contributors, who seem to be writing largely for themselves with little thought of readers, appear to be drawn exclusively from the ranks of the far left. To them modern science is "moulded by capitalist priorities" (Engels, quoted), and the British Labour Party is dangerously right-wing. Information technology is an instrument of militarists intent on nuclear war; those who support its development



suffer from a "fear of the persecuting breast" (a curious pseudo-freudian idea). Even the history of the piano is viewed from a marxist perspective, though "music almost always manages to escape classification and domination by political interests". Quite so.

Science does indeed need its critics, both for its own benefit and that of society at large. But the mixture of irrationality, intolerance and vituperation to be found in *Science as Culture* serves no purpose. □

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Journal prices

Details of editors and frequency of publication, and the subscription rates appearing at the top of each review, are given in most instances for 1990. This information is not complete in all cases. Readers interested in a particular journal should check prices before subscribing.

PR people in AI

P.W. Hawkes

International Journal of Pattern Recognition and Artificial Intelligence. Editors-in-charge H. Bunke and P.S.-P. Wang. *World Scientific*. 4/yr. \$260 (institutional); \$138 (personal).

THERE is no shortage of journals catering for the image-processing and pattern-recognition (PR) community: an IEEE series, the long-established and beautifully produced *Computer Vision Graphics, and Image Processing*, *Pattern Recognition* and *Pattern Recognition Letters*, as

INTERNATIONAL JOURNAL OF
PATTERN RECOGNITION AND
ARTIFICIAL INTELLIGENCE

well as specialized journals in medical imaging, remote sensing and the like. The list of artificial intelligence (AI) titles is not quite so long, but in neither field did one feel that important papers were piling up unpublished for want of journal space.

There was, however, no title that explicitly united AI and PR, and the object of this new journal is to attract papers in either field or, best of all, papers that involve both. Its success must be judged on this last category, for the remainder are already well catered for.

Rather to my surprise, there are a number of applied papers that do justify the existence of this new serial in the sense that they would otherwise have been published in journals so specialized that the PR and AI communities would probably have remained unaware of them. A good example is the contribution by H. Freeman and J. Ahn "On the Problem of Placing Names in a Geographic Map", a "seemingly simple task [that] is, in fact, remarkably subtle and complex". This is a fascinating article, with much to interest the student of image analysis, which no doubt would otherwise have appeared in *The American Cartographer* or *Annuaire International de Cartographie*.

Another vivid example is "Vision Applications in the Fishing and Fish Product Industries" by H. Arnarson, K. Bengoetxea and L.F. Pau, which tells us how machine vision can be used to detect worms (in cod...), bone residues and 'aesthetic' defects and also to sort different species despite the hostility of the ship-board environment. Here too, a glance at the list of references takes us outside the usual PR and AI territory with *Austral. Fish. J.*, *Prog. Fish. Cult.* and *J. Food Protection* among others.

A quotation will show why such a paper is of interest to those involved in pattern

recognition: "sorting by species as well as precise length estimation involves hypothesizing head and tail locations ... the filter kernels [for morphological filtering] ... must be size independent but species dependent. The shape anomalies [sic] between male and female fish as well as unretracted fins ... offer interesting challenges". Nevertheless, such papers as these are a small minority in the numbers I have seen, two of which contain conference proceedings (the Eighth International Conference on Pattern Recognition, held in Paris in 1986, and the International Workshop on Expert Systems, Novosibirsk, 1987).

Infant wisdom

G.F.J. Garlick

Physics World. Editor Philip Campbell. *Institute of Physics*. 12/yr. UK and elsewhere £53, North America \$104 (institutional); UK £23, North America \$44, elsewhere £25 (personal).

PHYSICS WORLD, an amalgamation and extension of two previous Institute of Physics' publications, began its monthly appearance in October of last year and is designed to present the interests of physics as a profession. The contents are wide ranging, running from the current problems of physics at all levels of education, through fiscal matters such as salaries and funding, to the interaction of British physicists with their counterparts in Europe and elsewhere, and reviews of the science itself.

To quote from the first editorial, the journal appeared "at a pivotal moment for physics, and in the long term, technological exploitation in the UK". Several issues subsequently describe the turmoil caused by new schools' curricula proposals from the Department of Education and Science, the critical-size test for university physics departments of the Edwards Committee and the faltering steps of the British government over continued support for CERN and high-energy physics. Again quoting the editor, "perhaps *Physics World* can be granted the privilege of the new born to express outrage at a hostile environment".

On a more positive note, most of the contents are devoted to review articles, both brief and extended. These generally deal with the frontiers of physics, or are sometimes historical, and all are written to avoid mathematical presentation (no mean feat given some of the topics). The standard is high and the publishers are to be commended in maintaining it over so many issues. Titles are sometimes snappy — "Quark Soup for Starters" or "Is Gravity as Simple as We Thought?" — but

The journal is well produced on glossy paper, accepts colour artwork and appears willing to print long articles. Publication times range from eight months to over a year and there are no page charges. The personal subscription rate does not seem excessive, unlike the inflated institutional rate. If the *International Journal of Pattern Recognition and Artificial Intelligence* is not indispensable, nor is it otiose — and if you cannot afford it, it is covered in *Current Contents*. □

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one does get in-depth treatment. Topical issues such as 'cold fusion' have been discussed soberly, much attention being paid to the weighing of claims against the evidence. A different but very satisfying combination was seen in the April 1989 issue, which was largely devoted to "Physics in Scotland". The range and intensity of physics activity there was certainly a revelation to me.

Physics and the activities of physicists need to be explained to a public which often sees only the menace of nuclear waste and weapons, and fails to realize how every corner of life is filled with the products of physics. This new publication will be a valuable medium for communication of this kind. There have already been articles on "Communications Breakdown in Nuclear Energy" and "Nuclear Power is Green Power" which make a significant contribution in this respect.

The section of *Physics World* that always occupies the last page of text is entitled "Lateral Thoughts". This is the Parthian shot, where levity is introduced.



The topics form delightful light reading and apparently are most popular among physics student finalists, who have received free copies during the last academic session. I hope they didn't skip too many of the pages to get to them. Particularly to be commended are the book reviews, which show just what can be achieved in this medium.

Each issue contains plenty of news about the Institute of Physics and its members, and you can also look for jobs or supplies of apparatus in the classified section. Finally, cost — for members, *Physics World* is especially cheap, but it is not at all exorbitant for libraries and others. □

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Dynamic model

John Guckenheimer

Nonlinearity. Honorary editors J.D. Gibbon and D.A. Rand. *Institute of Physics and London Mathematical Society.* 4/yr. UK £178, North America \$338. In the United States distributed by the American Institute of Physics.

THE joint sponsorship of *Nonlinearity* (by the London Mathematical Society and the Institute of Physics) is unusual and it bespeaks the vitality of research on 'non-linear' phenomena. The journal has already found itself a niche and it will undoubtedly persist as a publication of high quality. Its editorial board is strong and large enough to cover well the topics within its mandate — "nonlinear mathematics, mathematical and experimental physics and other areas in the sciences where nonlinear phenomena are of fundamental importance".

As the complement of all things linear, nonlinearity hardly seems a cohesive discipline. Nonetheless, there is a diverse group of scientists and mathematicians with overlapping interests in the phenomena concerned. This group of people forms the constituency of the new journal, as well as of several others that have slightly different emphases, and their work gives form to the subject. Among the topics of papers printed in the first few issues of *Nonlinearity* are chaos, fractals, nonlinear partial differential equations, dynamical systems, solitons, singularities and twisters.

The centre of gravity of *Nonlinearity* is distinctly different to that of such related journals as *Physica D*, *Ergodic Theory and Dynamical Systems*, *Communications of Mathematical Physics*, *Journal of Statistical Physics* and *Journal of Differential Equations*. *Physica D* is very similar in spirit to *Nonlinearity*, but its emphasis is decidedly more on physical aspects. On the other hand, *Ergodic Theory and Dynamical Systems* and *Journal of Differential Equations* are mathematics journals that frown on publishing papers that do not meet strict standards of mathematical rigour. In *Communications of Mathematical Physics* and the *Journal of Statistical Physics*, there is more emphasis upon models of statistical physics and field theories.

In *Nonlinearity* the stress is on dynamical systems theory. Over half of the papers published to date deal with the subject. There is a tremendous variation in the style of work in this area. At one end of the spectrum is pure mathematics with its insistence upon rigour, and at the other end is routine analysis of dynamical models that arise in many areas of science. The role of numerical studies and of theory that is founded upon careful

numerical study rather than rigorous proof has been a source of tension within the field. Although *Nonlinearity* includes many papers that would fit comfortably in other mathematical journals, it also serves the community of workers who rely upon computers. The blend of rigour and computationally justified theory found in *Nonlinearity* is refreshing, and I hope that it represents a trend that will spread to more areas of mathematics. □

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Chase for the holy grail

John Ruvalds

Physica C: Superconductivity. Editors M. B. Brodsky, G. W. Crabtree, B. D. Dunlap, R. P. Griessen, S. Maekawa, Yu. A. Osipyan, H. R. Ott and S. Tanaka. *North-Holland.* 24/yr. Dfl. 3,370.

Journal of Superconductivity. Co-editors Donald U. Gubser and Stuart A. Wolf. *Plenum.* 4/yr. North America \$135, elsewhere \$160 (institutional).

International Journal of Modern Physics B. Edited by the editorial board. *World Scientific.* 16/yr. \$485 (institutional); \$237 (personal).

Modern Physics Letters B. Edited by the editorial board. *World Scientific.* 22/yr. \$475 (institutional); \$234 (personal).

Superconductor Science and Technology. Honorary editor J. E. Evetts. *Institute of Physics.* 12/yr. £120, \$228.

EVER since the 1986 discovery of high-temperature superconductivity in an unlikely group of copper oxides, the race to find new materials with zero resistance to the flow of electrical current has generated immense excitement in the scientific community. The ultimate quest of a room-temperature superconductor has captivated thousands of chemists, physicists and a variety of other scientifically interested parties.

In addition to the intellectual challenge of understanding how the new superconductors function, the aura of a scientific 'breakthrough' and the promise of a new technology with imaginative commercial applications have attracted considerable interest among the general public, industrial representatives and even government officials. The dissemination of information in the field is thus beset by diverse demands.

The deluge of scientific data on the new superconductors has already surpassed 4,600 publications in a mere two years, and there is no sign of relief. Despite

progress in the growth of single crystal samples and a vast array of confirmed experimental features, the origin of the superconducting properties at high temperatures remains a mystery. Surprisingly, there is not even agreement on the nature of the charge carriers in the copper oxides. Holes, electrons and more exotic species have been proposed as viable candidates, but the crucial ingredient remains elusive. Then there is the dilemma of the ideal number of carriers needed to achieve superconductivity at the highest possible temperature, and the embarrassing existence of analogous cuprate compounds which do not become superconducting at all.

On the optimistic side, there now appear to be no crippling theoretical obstacles to superconductivity at room temperature, even though this dream was regarded as a fantasy only a few years ago. Of course, the lack of a definite mechanism for the cuprates does not constrain the enthusiasm and imagination of many researchers in the field.

To cope with the spectacular growth in the literature, many established journals have revised their refereeing procedures, putting the emphasis on rapid publication of novel results. The need for new specialist journals may thus be limited to filling a gap in the established coverage.

The new journal *Physica C: Superconductivity* is derived from the highly regarded *Physica*, which has a long tradition of publishing first-rate articles in the physical sciences. The editorial board is composed of international experts who are actively engaged in basic research in the field. The early issues are notable for breadth in coverage and overall high calibre of the scientific content. Theoretical contributions range from the fundamental issue of causality and the Josephson effect to a variety of computer calculations of electronic structures in specific materials. Several papers proposing innovative mechanisms for superconductivity have also appeared.

Experimental results published in *Physica C* include original findings on chemical substitutions which influence the transition temperature of the cuprate superconductors. Excellent articles on data for the heat capacity, transport properties, neutron scattering, nuclear spin resonance, critical magnetic fields, and microstructure characterization of samples attest to the high standard of this journal, which belongs in any library serving the basic research needs of scientists.

The *Journal of Superconductivity* aims to provide a new "forum for the publication of high-quality original articles on all aspects of the Science and Technology of Superconductivity". This ambitious goal has not been fully met in the first issues, but nevertheless there have been some interesting speciality features which may

mean the journal will fill a niche in a crowded field. For example Vol. 2, No. 1 consists of a superb bibliography of thousands of references compiled from a Westinghouse database. The index to keywords, authors, subjects and chemical element compositions is especially informative and useful. This source, in accessible, compact journal form, may well justify the cost of a trial subscription.

Featured articles in *Journal of Superconductivity* have included an outstanding, comprehensive review of heat-capacity measurements on cuprate compounds, and discussions of some technological applications. Contributions on magnetohydrodynamic ship propulsion, circuits, electron accelerators, logic devices and power applications are pitched at an introductory level, and should appeal to those involved in technology. The papers on sample growth and processing, with their exquisite colour photographs, exemplify the high quality of production. On average, the articles are brief and the first few issues do not pose a dire threat to library shelf space.

A committee of 39 distinguished scientists edits the *International Journal of Modern Physics B*, which covers condensed matter, statistical and applied physics. Another 28 experts oversee the section "Rapid Communications in High T_c Superconductivity". A broad geographical distribution of authors from Australia, Bahrain, Belgium, Brazil, Bulgaria and 19 other countries is evident in the handful of journals made available for review. In a similar vein, the supplement *Modern Physics Letters B* includes rapid communications and a section of "Brief Reviews" which exhibits short (typically three-page), less-technical summaries of select specialities.

Materials microfaults are the mainstay topic for *Superconductor Science & Technology*. Grain boundaries, oxygen defects and other imperfections need to be overcome for large-scale technology, and contributors examine the detailed morphology of samples in view of their influence on critical currents and other properties. Research on film-preparation techniques is neatly presented, with a critical examination of the prospects of the near-term applications in microwave detection and other signal-processing devices.

As the field matures, the emphasis on haste should yield to traditional values. It is a pleasure to find some interesting articles in all of the journals reviewed here. But their future success will be determined by the careful enforcement of standards, as well as by the setting of goals that complement rather than compete with those of established journals.

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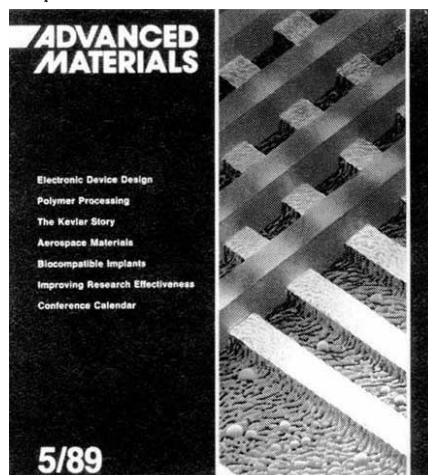
Communicating in a new era

Alan Windle

Materials Science and Engineering B: Solid-State Materials for Advanced Technology. Editors M. Balkanski, K. Kamimura and S. Mahajan. Elsevier Sequoia. 12/yr. SwFr.840.

Advanced Materials. Editor Peter Göltz. VCH. 12/yr. DM 298, £115, \$190 (institutional); DM 98, £42, \$79 (personal).

MATERIALS Science and Engineering B: Solid-State Materials for Advanced Technology was launched in August last year. If it hadn't been for the advent of high- T_c superconductivity, one wonders whether the publishers would have dared — a new



journal, in an otherwise fairly mature field which is already quite well provided for, is a risky venture.

Yet there is a clear professionalism about *Solid-State Materials*. It is a traditional, even classy, publication. One can play the name game, and try to guess its contents from the title, or perhaps from the editorial statement of scope inside the front cover. A better guide is the papers it has attracted to date, which deal almost exclusively with inorganic materials and concentrate most frequently on the relationship between structure and electronic properties. There is a good blend of well-established solid-state physics and solid-state chemistry, bound together by contributions which focus on the production of optical and electronic materials. The journal also provides a most appropriate environment for the 20 per cent or so of its papers which deal with the new superconductivity.

Authors seem to be treated well. The time between acceptance of papers and publication is averaging around six months, and there is every indication that this performance will be maintained. The quality of the reproduction of diagrams is high, and the micrographs come across

adequately, although they are usually disciplined into single columns where their comparatively small size means that they lack impact, if not clarity. There are also 25 reprints for free.

For 1989, the journal bills itself as a quarterly, although one can but puzzle over the numbering system. The rather thin issue of December 1988 is designated 3-4, whereas the first to appear this year (devoted to the proceedings of a conference on deep implants) was numbered 1-3, which meant that number 4 appeared in April! Indeed, the slightly bizarre numbering is associated with a lack of uniformity both in publication dates and the thickness of the offering. Of course this may settle with time, and any confusion will tend to be reduced on binding.

Here we have a solid and respectable journal.

Advanced Materials is a different type of publication altogether. It is a monthly, aimed at the materials scientist, whose subject has grown and diversified so dramatically over the past ten years. Although it is possible to be an expert in one corner of the discipline, there is the perennial need to remain informed across the whole field, and a need too to foster a professional identity. If journals are to unify materials research rather than segregate it into subdisciplines, they must somehow represent the various components without becoming unwieldy.

Advanced Materials seems to have found this niche. It is a heady mixture of essays, review and research communications, news and conference data, and it works admirably. The emphasis is on new materials, and there is a fair balance across the component subjects of metallurgy, ceramics, composites and polymers. It is perhaps the journal one would most like to have delivered to a busy desk — the articles can be read in a few minutes, yet they communicate at the highest level and reflect the perception of their often-distinguished authors. One can of course quibble at the presentation; the paper used is too thin, for example, the print showing through from the other side of each page.

There is currently some basic rethinking, among materials scientists at least, as to how research should best be published, and the German community is among the leaders in this respect. We may be seeing the end of the traditional research paper, which could be superseded by a combination of rapid communication of results from laboratory to print with periodic state-of-the-art reviews. *Advanced Materials* is a part of this new era, and that is perhaps the underlying reason why it is exciting. □

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On the third branch

Colin Upstill

Computers in Physics. Editor Robert R. Borchers. *American Institute of Physics.* 6/yr. US \$125, Canada and South America \$135, elsewhere \$140 (institutional); US \$25, Canada and South America \$35, elsewhere \$40 (individual).

In the distant past, physics was thought of as a branch of philosophy, and the behaviour of the physical world was understood purely by theorizing about it. The computerization of physics today is a revolution of similar magnitude to the experimentalization of physics that took place around the time of Galileo. In the history of physics, the 1980s will be remembered neither for high-temperature superconductivity, nor for cold fusion, but as the decade in which computers really entered the methodology of physics.

Computers in Physics is an interdisciplinary periodical based on the recognition that a third branch of science is rapidly developing. It also tries to combine learned journal with magazine, juxtaposing peer-reviewed archival papers with news, views and feature articles. Most of the latter are excellent, but the quality of the research papers is less certain: some are introductory, some expository, and few contain much that is really novel.

The range is vast. In early issues, subjects dealt with include expert systems, computer algebra, supercomputer algorithms, particle physics codes, colour vision, the fractal dimension of cerebral surfaces, seismic tomography, computer architectures, parallel processing (highly relevant, as so much computing in physics needs supercomputer performance), optical computing, neural networks, the physical limits of computing (physics is as unavoidable within the machines as the machines are in physics), and software construction methods. It is especially good to see coverage of this last topic — unlike chemists, physicists tend to write their own code, and are usually self-taught, with bad or even dangerous habits.

After an initial hiccup — there is but one paper, six pages long, in the 96 pages of the third issue — *Computers in Physics* has settled down to being almost exactly half magazine and half journal. There are regular columns on numerical recipes, simulations, computers in physics education and spreadsheets. Spreadsheets can be used for purposes far beyond their original applications in accounting, and offer a remarkable capability for quick and easy solutions to a broad range of scientific problems; sharp instruments cut

both ways, however, and these programs can provide the unwary with pretty collections of incorrect answers.

There is a fair amount that is good about *Computers in Physics*, and much that is mixed. The papers have lots of program listings in them, but many lines are irrelevant to the technical content of the paper; language-independent pseudo-code or flow diagrams are far better, and happily these have started to appear in the most recent issues. Colour is heavily used, but in the journal section it is largely negated by complicated pictures being reproduced at the size of postage stamps.

The articles and papers have a reasonably international authorship, though with a decided US bias which is both understandable and acceptable. Yet apart from a couple of brief references to parallel-processing activities in Europe and to Japanese supercomputers (and in terms which imply 'the threat from'), the news pages are almost entirely US-orientated. In consequence the international appeal is considerably diminished, as is the value of

the periodical to its US readers. Another failing is that there is no obvious distinction between the magazine and journal pages. To be concerned at this is not elitism — the boundary conditions on an author are different for magazine articles and for peer-reviewed papers, and the extent to which another researcher can rely on each varies accordingly.

Communications of the ACM, *Computer* and *IEEE Software* have most of the good points of *Computers in Physics*, such as colour illustrations, and cover between them its computing aspects. With *American Journal of Physics* or *European Journal of Physics* for more pedagogical papers, and the established physics literature for specific applications, the considerable range of *Computers in Physics* is already well catered for. The question is whether or not it is worthwhile to have such diverse material in one place. I rather doubt it. □

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Also submitted for review

The following list shows the journals that were eligible for review but that are not covered in the preceding pages. It does not include those titles that had not published enough issues to be considered (see page 350).

Acta Geologica Sinica (Geological Publishing House, Beijing, distributed by Pergamon).

Acta Meteorologica Sinica (China Meteorological Press, Beijing, distributed by Pergamon).

American Journal of Hypertension: Journal of the American Society of Hypertension (Elsevier).

APMIS (formerly *Acta Pathologica, Microbiologica et Immunologica Scandinavica*) (Munksgaard, Copenhagen).

Australian Systematic Botany (CSIRO/Australian Academy of Science, East Melbourne, Victoria).

Cellular Signalling (Pergamon).

Chinese Journal of Atmospheric Sciences (Allerton, New York).

Chinese Journal of Infrared and Millimeter Waves (Allerton, New York).

Clinical Microbiology Reviews (American Society for Microbiology).

Experimental Thermal and Fluid Science: International Journal of Experimental Heat Transfer, Thermodynamics, and Fluid Mechanics (Elsevier).

International Journal of Numerical Modelling: Electronic Networks, Devices and Fields (Wiley).

Journal of Planar Chromatography — Modern TLC (Hüthig).

Journal of Food Composition and Analysis: An Official Publication of the

United Nations University International Network of Food Data Systems (Academic).

Journal of Human Nutrition and Dietetics: The Official Journal of the British Dietetic Association (Blackwell Scientific).

The Journal of Trace Elements in Experimental Medicine (Alan R. Liss).

Microbial Ecology in Health and Disease (Wiley).

Microwave and Optical Technology Letters (Wiley).

Muon Catalyzed Fusion: Journal Devoted to Theory and Experiment in μ CF and Its Applications, Mesic Atomic and Molecular Physics (Baltzer, Basel).

The National Medical Journal of India (All India Institute of Medical Sciences, New Delhi/Oxford University Press).

Nuclear Geophysics: A Journal of Nuclear Techniques in the Earth and Environmental Sciences, Minerals Exploration, Mining and Process Control (Pergamon).

Pigment Cell Research (Alan R. Liss).

Reproductive and Genetic Engineering: Journal of Feminist Analysis (Pergamon).

Reproductive Toxicology (Pergamon).

Resources, Conservation and Recycling (incorporating *Resources and Conservation and Conservation and Recycling*) (Elsevier/Pergamon).

Schizophrenia Research: An International Multidisciplinary Journal (Elsevier).

Soviet Journal of Applied Physics: Thermophysics, Heat Transfer, Plasma Physics, Protective Coatings, Fluid Dynamics, Cold Climate Engineering (Scripta Technica, a subsidiary of Wiley).